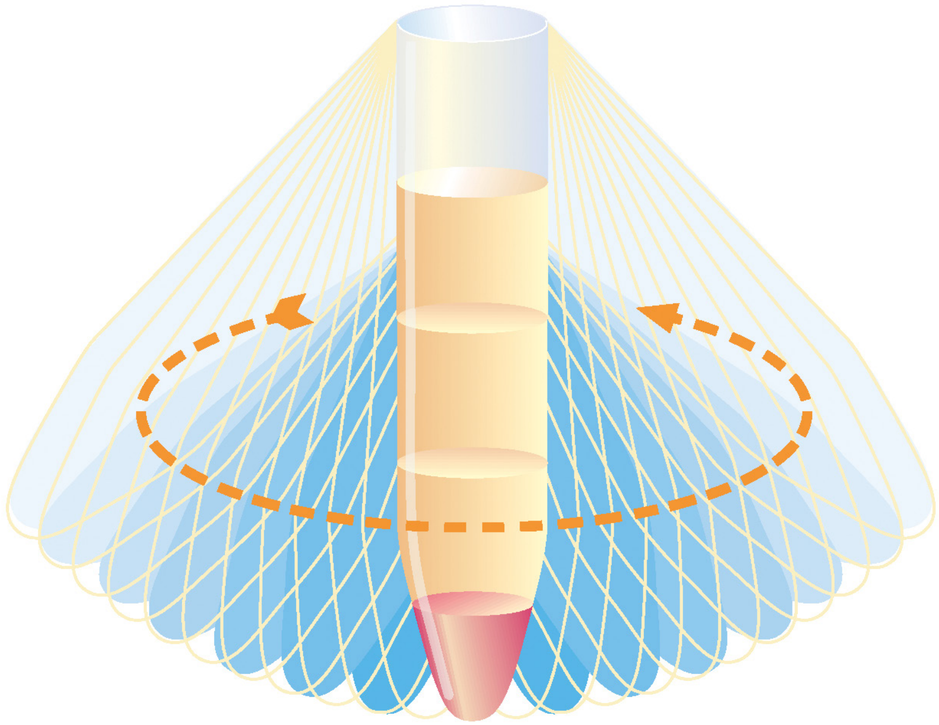
Methodology and applications

Cell separation media



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Contents

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Introduction

Since its introduction in 1977, the silica colloid Percoll™ has become the density gradient medium of choice for thousands of researchers worldwide. Its nearly ideal physical characteristics facilitate its use in separating cells, organelles, viruses, and other subcellular particles. Percoll is especially useful as a first step to enrich for cell populations before attempting finer resolution or extraction of nucleic acids. A considerable savings of time and resources may be realized using Percoll as a first step before employing these methods.

For biological particles, the ideal gradient medium has been described as one having the following characteristics (79):

- covers a sufficient density range for isopycnic (Fig 1) banding of all biological particles of interest
- possesses physiological ionic strength and pH
- is iso-osmotic throughout the gradient
- has low viscosity
- is non-toxic
- will not penetrate biological membranes
- will form self-generated gradients by centrifugation at moderate g-forces
- is compatible with biological materials
- is easily removed from purified materials
- does not affect assay procedures
- will not quench radioactive assays

Percoll is exceptional among the available media in that it fulfills the above criteria, and also provides these additional advantages:

- It can form both continuous and discontinuous gradients
- Stability of gradients means that gradients can be premade to give reproducible results
- Analysis of gradients is simple with colored Density Marker Beads (available from Cospheric LLC, USA)
- Further experiments with isolated materials are not affected by Percoll
- The success of thousands of researchers has been documented in the Percoll Reference List

This re-issued manual provides the basic methodology for making and using gradients of Percoll and includes information on Percoll PLUS, a new silica based colloidal medium optimized for cell separation in clinical research applications. In addition, the Application Tables in the latter part of this manual provide numerous references for using Percoll to isolate various cells, microorganisms, organelles and subcellular particles. All experiments described in the literature using Percoll can also be performed with Percoll PLUS.

Principles of density gradient centrifugation

When a suspension of particles is centrifuged, the sedimentation rate of the particles is proportional to the force applied. The physical properties of the solution will also affect the sedimentation rate. At a fixed centrifugal force and liquid viscosity, the sedimentation rate is proportional to the size of the particle and the difference between its density and the density of the surrounding medium.

The equation for the sedimentation of a sphere in a centrifugal field is:

$$v = \frac{d^2 (\rho_p - \rho_l)}{18\eta} \times g$$

where
- v = sedimentation rate
- d = diameter of the particle (hydrodynamically equivalent sphere)
- ρ_p = particle density
- ρ_l = liquid density
- η = viscosity of the medium
- g = centrifugal force

From this equation, the following relationships can be observed:

- The sedimentation rate of a particle is proportional to its size
- The sedimentation rate is proportional to the difference between the density of the particle and that of the surrounding medium
- The sedimentation rate is zero when the density of the particle is equal to the density of the surrounding medium
- The sedimentation rate decreases as the viscosity of the medium increases
- The sedimentation rate increases as the centrifugal force increases

Separation by density (isopycnic centrifugation)

In this technique, the density range of the gradient medium encompasses all densities of the sample particles. Each particle will sediment to an equilibrium position in the gradient where the gradient density is equal to the density of the particle (isopycnic position). Thus, in this type of separation, the particles are separated solely on the basis of differences in density, irrespective of size.

Figure 1 illustrates the two types of centrifugal separation (see below for rate zonal centrifugation). When using Percoll, it is common to separate particles isopycnically rather than on the basis of size differences (but see Figure 19, page 31, where both techniques are used.).

Note: When considering biological particles, it is important to remember that the osmolality of the medium can significantly alter the size and apparent buoyant density of membrane-bound particles. A high external osmolality will cause membrane-bound particles to shrink while a low osmolality in the medium will cause the particles to swell.

Figure 2 shows that particles centrifuged in gradients of Percoll under physiological conditions (280 to 320 mOsm/kg H_2O) have much lower apparent buoyant densities than in sucrose or metrizamide (see also Table 1, page 16).

Separation by size (rate zonal centrifugation)

In this type of separation, the size difference between particles affects the separation along with the density of the particles. As can be seen from the above equation, large particles move faster through the gradient than small particles, and the density range is chosen so that the density of the particles is greater than the density of the medium at all points during the separation (Fig 1). The run is terminated before the separated zones reach the bottom of the tube (or their equilibrium positions).

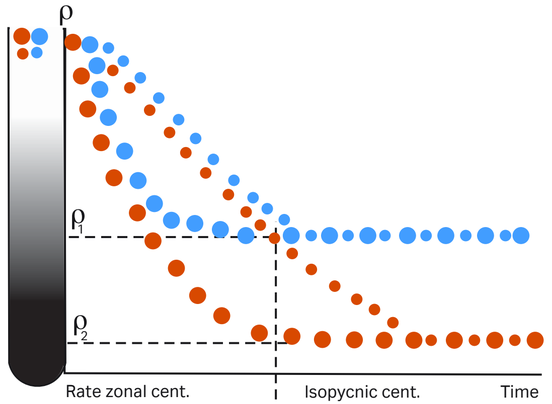


Fig 1. Diagrammatic representation of rate zonal and isopycnic centrifugation.
ρ_1 = buoyant density of the less dense (blue) particles
ρ_2 = buoyant density of the more dense (red) particles
(Courtesy of H. Pertoft, reproduced by kind permission).

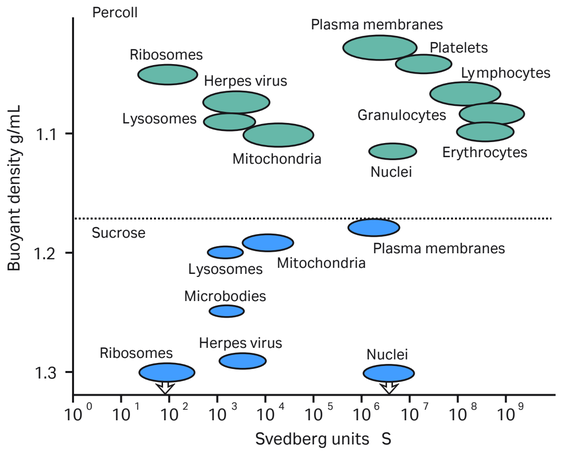


Fig 2. Approximate sedimentation rates and isopycnic banding densities of particles in a rat liver homogenate, herpes virus and human blood cells in gradients of Percoll (green) compared with sucrose gradients (blue). Svedberg units = sedimentation coefficient, 1S = 10^{-13} s. (27, reproduced by kind permission of the authors and publisher).

Percoll – physical properties

Percoll is available from Cytiva.	
Composition	silica sol with nondialyzable polyvinylpyrrolidone (PVP) coating
Density	1.130 ± 0.005 g/mL
Conductivity	1.0 mS/cm
Osmolality	< 25 mOsm/kg H_2O
Viscosity	10 ± 5 cP at 20°C
pH	9.0 ± 0.5 at 20°C
Refractive Index	1.3540 ± 0.005 at 20°C
Percoll is non-toxic	

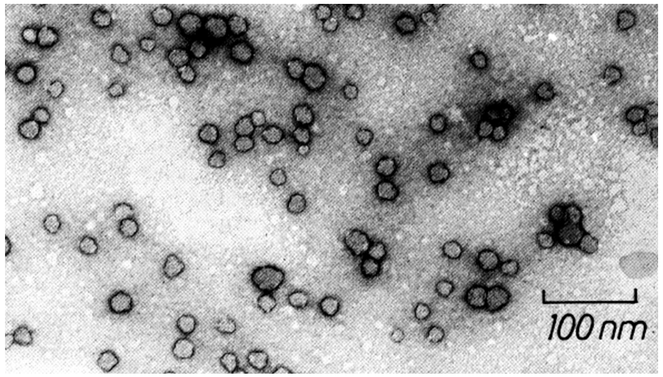


Fig 3. Electron microscopy of Percoll particles. Negative contrast with 1% uranyl acetate at pH 4.6 (21, reproduced by kind permission of the authors and publisher).

Particle size composition

The physical properties of Percoll have been extensively studied by Laurent *et al.* (45, 46, 47). Electron microscopic examination (Fig 3) shows the silica to be in the form of a polydisperse colloid composed of particles from 15 to 30 nm in size, with a mean particle diameter of 21 to 22 nm. Hydrodynamic measurements (viscometry and sedimentation) give values of 29 to 30 nm and 35 nm in 0.15 M NaCl and water, respectively, for the mean particle diameter, indicating a layer of hydration on the particles which is more pronounced at low ionic strength.

Chromatography of Percoll on Sepharose™ 4B (22) has demonstrated the presence of only 1 to 2% free PVP. Inclusion of PEG in the eluant did not result in any loss of PVP from the silica, indicating that the PVP is firmly bound. Calculations based on the nitrogen content of the colloid indicate that the PVP coating is a monomolecular layer.

Viscosity

The viscosity of Percoll is a function of the ionic strength, and is lower in saline solutions at physiological ionic strength (e.g., 0.15 M NaCl) than in water or in 0.25 M sucrose (22).

This has the effect of making gradient formation in 0.15 M NaCl much faster than in 0.25 M sucrose when solutions are centrifuged under identical conditions (page 17). Under working conditions, the viscosity of Percoll solutions is 1 to 15 cP, facilitating extremely rapid banding of particles in gradients of Percoll.

Density

Percoll is supplied as a 23% (w/w) colloidal solution in water having a density of 1.130 ± 0.005 g/mL.

Gradients ranging from 1.0 to 1.3 g/mL are achievable by centrifugation as described elsewhere in this booklet. All biological particles having sedimentation coefficient values of > 60S can be successfully banded on gradients of Percoll, and most have buoyant densities of < 1.13 g/mL in Percoll (Fig 2).

pH and osmolality

Percoll has a pH of about 9.0, adjustable to pH 5.5 to 10.0 without any change in properties. If the pH is dropped below 5.5, gelling may occur. Gelling can also be caused by the presence of divalent cations, an effect which is exacerbated by elevated temperatures.

Percoll has a very low osmolality (< 25 mOsm/kg H_2O) and can therefore form a density gradient without producing any significant osmolality gradient itself. This makes it possible to work with density gradients which are iso-osmotic and adjusted to physiological conditions throughout. This is very important for obtaining preparations of cells having extremely high viabilities (23), and intact morphology (31). Due to this fact, gradients of Percoll also provide an opportunity to observe the effect of osmolality on the apparent buoyant density of cells and subcellular particles (see page 16 and ref. 27).

Behavior of the colloid

Percoll particles have an inner core of silica which is very dense (ρ = 2.2 g/mL) and an average hydrated particle size of 29 to 30 nm in 0.15 M NaCl and 35 nm in water (46). Thus, when a solution of Percoll (in 0.15 M saline or 0.25 M sucrose) is centrifuged at > 10 000 × g in an angle-head rotor, the coated silica particles will begin to sediment. This results in an uneven distribution of particles, and thus forms a density gradient. Since Percoll is a polydisperse colloid, its component particles will sediment at different rates, creating a very smooth gradient. Electron microscopic analysis of gradients by high speed centrifugation in an anglehead rotor shows that the material at the bottom of the tube is considerably enriched in larger particles (Pertoft, personal communication). The gradient forms isometrically (i.e., less dense on top and more dense on the bottom) around the starting density and becomes on average progressively steeper with time (Fig 4). Prolonged centrifugation of Percoll at high g-forces results in all the colloid sedimenting to form a hard pellet (see *Removal of Percoll*, page 26). It is important to note that if a gradient of Percoll is spun at > 10 000 × g in a swinging-bucket type rotor, the colloid will rapidly sediment into a pellet and not form a suitable gradient.

The colloid does not perceptibly diffuse over time, resulting in the formation of very stable gradients. Therefore, both discontinuous and continuous gradients can be prepared weeks in advance, giving great reproducibility over the course of an experiment.

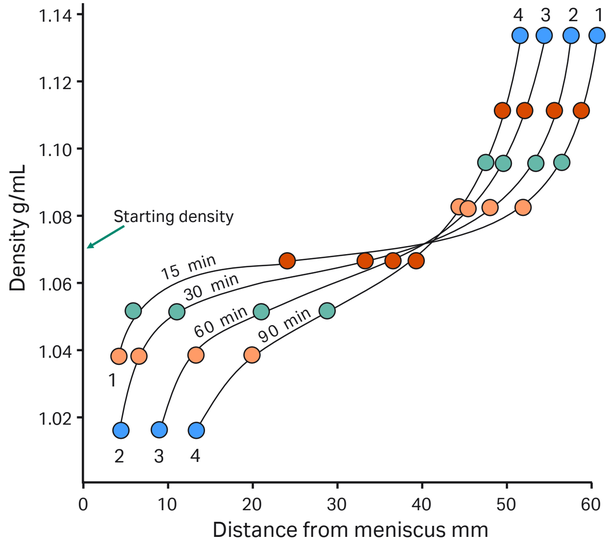


Fig 4. Isometric gradient formation by Percoll in an angle-head rotor, 8 × 14 mL (MSE Superspeed centrifuge) starting density 1.07 g/mL in 0.15 M NaCl. Running conditions: 20 000 × g for 15, 30, 60 and 90 min. Gradient density was monitored by means of colored Density Marker Beads (Fig 12) (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

How to make and use gradients of Percoll

Making and diluting a stock solution of Percoll

In order to use Percoll to prepare a gradient, the osmolality of Percoll (undiluted) must first be adjusted with saline or cell culture medium to make Percoll isotonic with physiological salt solutions. Adding 9 parts (v/v) of Percoll to 1 part (v/v) of 1.5 M NaCl or 10× concentrated cell culture medium is a simple way of preparing a Stock Isotonic Percoll (SIP) solution. Final adjustment to the required osmolality can be carried out by adding salts or distilled water. Cell density depends on osmolality (Fig 5); because of this, the osmolality of the stock solution should be checked routinely with an osmometer to ensure reproducibility between experiments. For subcellular particles which aggregate in the presence of salts, the Stock Isotonic Percoll (SIP) can be made by adding 9 parts (v/v) of Percoll to 1 part (v/v) of 2.5 M sucrose.

The density of the SIP solution can be calculated from the following formula:

$$V_x = V_o \frac{(\rho_o - \rho_i)}{(\rho_i - \rho_{10})} \qquad \text{thus } \rho_i = \frac{V_o\rho_o + V_x\rho_{10}}{V_x + V_o}$$

where V_x = volume of diluting medium (mL)

V_o = volume of undiluted Percoll (mL)

ρ_o = density of Percoll (1.130 + 0.005 g/mL*)

ρ_{10} = density of 1.5 M NaCl = 1.058 g/mL (minor differences for other salts)
density of 2.5 M sucrose = 1.316 g/mL (minor differences for other additives)

ρ_i = density of SIP solution produced (g/mL)

Thus, for SIP in saline, ρ_i = 1.123 g/mL and for SIP in sucrose, ρ_i = 1.149 g/mL, assuming ρ_o = 1.130 g/mL.

* Exact density as stated on the Certificate of Analysis, which can be found under *Literature* at www.cytiva.com.

Diluting stock solutions to lower densities

Solutions of Stock Isotonic Percoll (SIP) are diluted to lower densities simply by adding 0.15 M NaCl (or normal strength cell culture medium) for cell work, or with 0.25 M sucrose when working with subcellular particles or viruses.

The following formula can be used to calculate the volumes required to obtain a solution of the desired density.

$$V_y = V_i \frac{(\rho_i - \rho)}{(\rho - \rho_y)}$$

where V_y = volume of diluting medium in mL

V_i = volume of SIP in mL

ρ_i = density of SIP in g/mL

ρ_y = density of diluting medium in g/mL
(density of 0.15 M NaCl is ~1.0046 g/mL) *
(density of 0.25 M sucrose is ~1.032 g/mL)*

ρ = density of diluted solution produced in g/mL

Example: To dilute 55 mL of SIP to a final density of 1.07 g/mL, determine the amount of 0.15 M NaCl required.

$$\text{Volume of 0.15 M NaCl required} = 55 \times \frac{1.123 - 1.07}{1.07 - 1.0046}$$
$$= 44.6 \text{ mL}$$

The above formula is useful for achieving densities that will be very close to the actual density desired. However, slight variations in volumes and densities of diluting media will affect final density. For determining actual densities, we recommend measuring the final density of Percoll solutions using a densitometer or refractometer (see page 24).

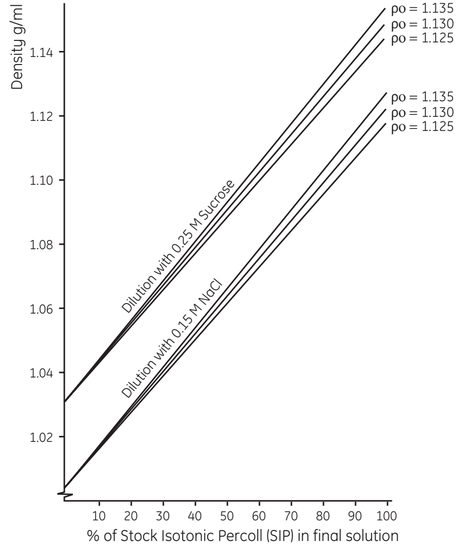


Fig 5. Dilution of Stock Isotonic Percoll (SIP) with iso-osmotic saline or sucrose solution. Po is the density of the Percoll (undiluted). SIP is prepared as described on page 12. The calibration lines shown are for guidance only. For accurate density measurements, refer to the formula given in the text (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

Note: The graph shown in Figure 5 can also be used as an empirical guide to the density of solutions produced by diluting SIP with 0.15 M saline or 0.25 M sucrose. This graph refers to the dilution of SIP where SIP is 90% (v/v) undiluted Percoll osmotically adjusted by addition of 10% (v/v) saline or sucrose. To avoid confusion, it is therefore preferable to refer to the actual density of the working solution (or to state % SIP) rather than to refer to the solution as a percentage of Percoll in iso-osmotic saline or sucrose. This is particularly important when using the one-step dilution procedure described below, where a working solution of known density is obtained by diluting Percoll (undiluted) plus concentrated salts or sucrose to a final volume with distilled water.

* from CRC Handbook of Chemistry and Physics, 67th edition (1986-1987), CRC Press, D253 and D262.

The one-step procedure for diluting Percoll

Percoll (undiluted) may de diluted directly to make a final working solution of known density by the following procedure. In a measuring cylinder, add 1.5 M NaCl or 2.5 M sucrose to 1/10 of the final desired volume (e.g., 10 mL for 100 mL of working solution). To this, add the required volume of Percoll (undiluted), calculated using the formula shown below. Make up to the final volume with distilled water.

$$V_o = V \frac{\rho - 0.1\rho_{10} - 0.9}{\rho_o - 1}$$

where V_o = volume of Percoll (undiluted) (mL)

V = volume of the final working solution (mL)

ρ = desired density of the final solution (g/mL)

ρ_o = density of Percoll (undiluted) (g/mL)
(see Certificate of Analysis for exact density)

ρ_{10} = density of 1.5 M NaCl = 1.058 (g/mL)
(minor differences for other salts)
density of 2.5 M sucrose = 1.316 (g/mL)
(minor differences for other additions)

Example: To prepare 100 mL of working solution of Percoll of density 1.07 g/mL in 0.15 M NaCl. To 10 mL of 1.5 M NaCl, add

$$\text{Volume of Percoll required} = 100 \times \frac{1.07 - 0.1058 - 0.9}{0.13}$$

= 49.4 mL (if Percoll density is 1.130 g/mL) and make up to 100 mL with distilled water.

The above formula is useful for achieving densities that will be very close to the actual density desired. However, slight variations in volumes and densities of diluting media will affect final density. For determining highly accurate densities, we recommend measuring the final density of Percoll solutions using a densitometer or refractometer (see page 24).

Graphs similar to the one shown in Figure 5 can be drawn to relate the volume of Percoll (undiluted) to the final density.

Diluting Percoll to a desired osmolality

To make isotonic Percoll for most mammalian cells, it is common to dilute 9 parts of Percoll (undiluted) with 1 part of 1.5 M NaCl or 2.5 M sucrose solution. This Stock Isotonic Percoll (SIP) is then further diluted with physiological buffers according to needs. However, while this procedure has proved successful, it is rather simplistic and does not take into account the effect of having solid silica particles present (i.e., that 100 mL of Percoll stock contains a certain volume of solid silica, making the total aqueous volume less than 100 mL). Due to the volume occupied by silica, the electrolytes in the stock solution have a higher effective concentration than in physiological salt solution, and SIP made in this way will be hyperosmolal. Thus, determining the actual osmolality of the SIP has always been recommended.

Vincent and Nadeau (555) discuss the problem elegantly and described an equation which can be used to calculate the number of parts of Percoll which should be added to one part of 10× concentrated physiological salt buffer to obtain a SIP of any desired osmolality. The authors determined the fraction of the total volume of a Percoll stock solution which is occupied by silica and thus determined the ratio of volume of aqueous solution to that of total Percoll stock solution.

$$V_p = V_c \frac{O_c - O_f}{R(O_f - O_p)}$$

where V_p = number of parts of Percoll to be added

V_c = number of parts of solute concentrate (e.g., 1.5 M NaCl) to be added

O_c = osmolality of solute concentrate (e.g., 1.5 M NaCl = 2880 mOsm)

O_f = desired osmolality

R = ratio of aqueous volume to total volume of Percoll (typically = 0.85 for NaCl and 0.80 for sucrose)

O_p = osmolality of Percoll undiluted (see Certificate of Analysis)

The key variable in this equation is R, which is a measure of the real aqueous volume of a Percoll solution. The value of R is a function of the hydrodynamic volume occupied by the Percoll particles. This, in turn is a function of the ionic strength of the medium; that is, as ionic strength increases, hydrodynamic volume decreases. Thus, there is a difference in the R value of 1.5 M NaCl and 2.5 M sucrose.

To obtain a SIP of osmolality = 320 mOsm/kg H_2O adjusted with 1.5 M NaCl (i.e., 10× concentrated physiological saline):

$$V_p = 1 \frac{2880 - 320}{0.85\ (320 - 20)} = 10.04$$

assuming: 2880 = osmolality of 1.5 M NaCl (10× concentrated physiological saline)
20 = osmolality of Percoll undiluted

Therefore to obtain a SIP of 320 mOsm/kg H_2O, one would add 10 parts Percoll to 1 part 1.5 M NaCl.

The ratio of concentrated solute solution (i.e., 1.5 M NaCl, etc.) to SIP is called Rx where:

$$R_x = \frac{V_c}{V_p + V_c}$$

Using this formula, one can calculate the amount of Percoll (undiluted) required to make a final working solution of known density and osmolality.

$$V_o = V \frac{\rho - R_x\rho_{10} - (1-R_x)}{\rho_o - 1}$$

where V_o = Volume of Percoll undiluted (mL)

V = Volume of final working solution (mL)

ρ = desired density of final working solution (g/mL)

R_x = fraction of total volume which is solute concentrate (i.e., 1.5 M NaCl, etc.)

ρ_o = density of Percoll undiluted (g/mL)
(see Certificate of Analysis)

ρ_{10} = density of 1.5 M NaCl (1.058 g/mL), 2.5 M sucrose (1.316 g/mL), etc.

Thus, for 100 mL of SIP of osmolality = 320 mOsm/kg H_2O adjusted with NaCl and density = 1.07 g/mL:

$$Vo = 100 \frac{1.07 - 1/11 \times 1.058 - (1-1/11)}{1.13 - 1} = 49.8 \text{ mL}$$

The final solution contains 9.1 mL of 1.5 M NaCl (1/11 × 100 = 9.1), 49.8 mL Percoll undiluted and 41.1 mL (i.e., 100 - 58.9 = 41.1) of distilled water.

Effects of osmolality on apparent buoyant density of cells and subcellular particles

The very low osmolality of Percoll has facilitated the study of the interrelation of the separation medium osmolality with the apparent buoyant density of particles. Figure 6 shows the effects of banding rat liver hepatocytes in Percoll gradients having osmolalities of 200, 300 and 400 mOsm/kg H_2O. The apparent buoyant density of the cells increases with increasing osmolality, due to removal of water from the cells. The same effect has been observed with mitochondria (Fig 7) and with lysosomes (Table 1). Even small changes in osmolality cause a large change in the apparent buoyant densities of these organelles. The actual recorded buoyant densities of particles banded in Percoll gradients at physiological osmolality are therefore much more likely to correspond to those existing *in vivo*, than when the particles are banded in sucrose or other centrifugation media.

Table 1. Changes in buoyant density of lysosomes after incubation in serum albumin

Incubation medium		Osmolality of medium	Average density of
Albumin %	Sucrose %	(mOm/l)	lysosomes (g/mL)
-	8.5	284	1.045
2.5	8.5	288	1.058
5	8.5	292	1.074
7.5	8.5	300	1.078
10	8.5	310	1.091
20	8.5	374	1.110
30	8.5	503	1.148
40	8.5	800	1.177

A lysosomal fraction from rat hepatocytes was recovered from a Percoll/0.25 M sucrose gradient at a density of 1.0 to 1.05 g/mL and incubated in the media described in the table for 1 h at 37°C. The buoyant density was then redetermined in a gradient of Percoll/0.25 M sucrose (27, reproduced by kind permission of the authors and publisher).

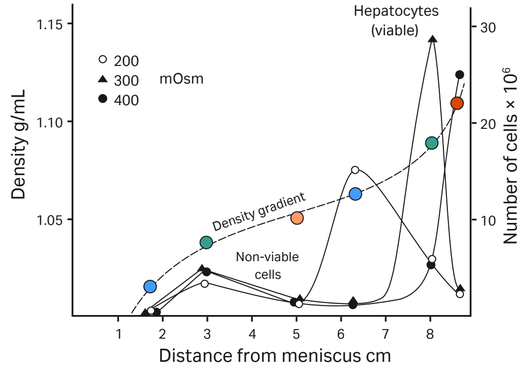


Fig 6. Fractionation of rat liver hepatocytes cells (35 × 10^6 cells in a volume of 2 mL) on a self-generated Percoll gradient (8 mL solution with a density of 1.065 g/mL). The osmolality of the Percoll solution was varied by adding NaCl to 200 mOsm, 300 mOsm and 400 mOsm. Centrifugation was performed in a Beckman rotor 30.2 for 15 min at 35 000 × g at a temperature of 4°C. Density gradient determined using Density Marker Beads (see page 22) (27, reproduced by kind permission of the authors and publisher).

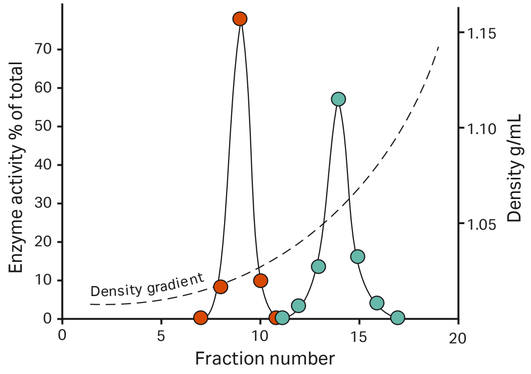


Fig 7. The density distribution of mitochondria from rat liver cells after incubation in iso-osmotic buffer (orange) and buffer containing 17.5% albumin (green). Centrifugations were performed in a Beckman 65 rotor (23° angle) for 30 min at 40 000 × g (59, reproduced by kind permission of the authors and publisher).

Factors affecting gradient formation and shape

Although the hydrated volume of Percoll particles is smaller in the presence of 0.15 M NaCl than in Percoll/0.25 M sucrose, the sedimentation rate of the particles is faster due to the lower viscosity of Percoll in saline. Thus, when Percoll is made iso-osmotic with a final concentration of 0.15 M saline or a tissue culture medium of equivalent ionic strength, it will form a self-generated gradient about 2 to 3 times faster than the equivalent Percoll solution made iso-osmotic with a final concentration of 0.25 M sucrose.

Centrifugation and time are interrelated in that it is the total (g-force) × (time) which determines the shape of the gradient. A minimum of approximately 10 000 × g should be used for Percoll in 0.15 M saline and about 25 000 × g for Percoll in 0.25 M sucrose in order to self-generate gradients in anglehead rotors. Rotor geometry has a marked effect on gradient shape under given conditions as shown in Figure 8. As the angle approaches vertical, the pathlength for formation of the gradient becomes shorter and the gradient forms more rapidly. Figures 9 and 10 demonstrate that the initial concentration of Percoll also has some effect on the shape of the gradient formed.

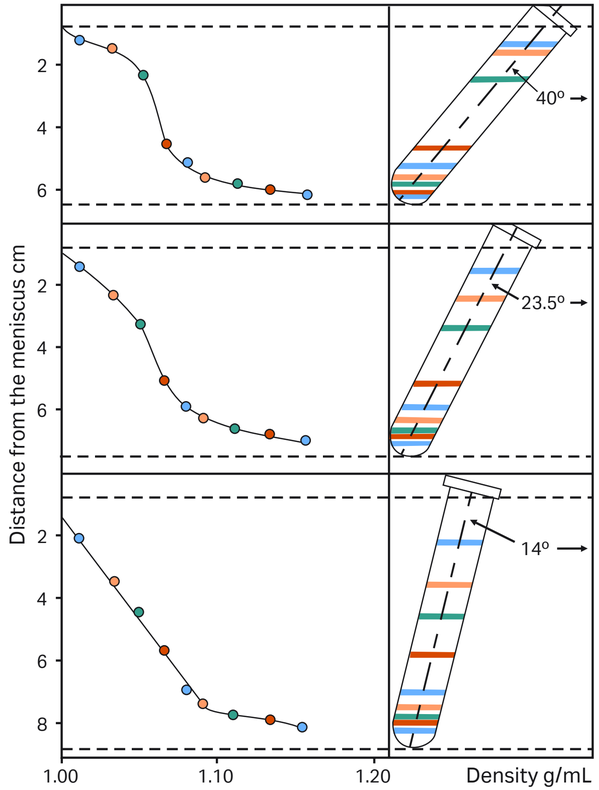


Fig 8. The effect of rotor angle on gradient development using Percoll. Starting density was 1.065 g/mL in 0.15 M NaCl. Running conditions: 30 000 × g for 14 min. Colored lines refer to the positions of the colored Density Marker Beads (45, reproduced by kind permission of the authors and the publisher).

Centrifugation in vertical rotors will form gradients of Percoll very rapidly. Care must be taken, however, to ensure that the compacted pellet of Percoll which may be formed under high speed centrifugation conditions does not contaminate the gradient during fractionation.

The use of swinging bucket rotors for self-generation of gradients is not recommended, due to the long path length and unequal g-force along the tube. However Jenkins *et al.* (personal communication and ref. 87) report some advantages in using these types of rotors for subcellular fractionation of liver organelles.

Zonal rotors can be used to form gradients of Percoll *in situ.* Gradients formed in zonal rotors have the same characteristics as those generated in angle-head rotors. Because of their large sample volumes, it is recommended that the separation conditions in a nonzonal rotor be empirically determined prior to scale-up in a zonal rotor. Zonal rotors have been used in the large scale purification of viruses (21) and for subfractionation of lysosomes (24).

When starting work with self-generated gradients, it is advisable to conduct a model experiment with colored Density Marker Beads (see page 21) to produce a series of standard curves under known conditions which are characteristic of the angle-head rotor to be used for subsequent experiments.

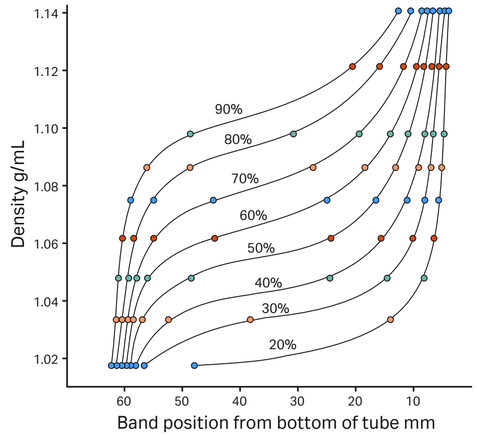


Fig 9. Use of colored Density Marker Beads to show gradient shape. Gradients formed from solutions of Percoll varying from 90% to 20% of stock isotonic Percoll in 0.15 M NaCl. Running conditions 23° angle-head rotor 30 000 × g, 15 min (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

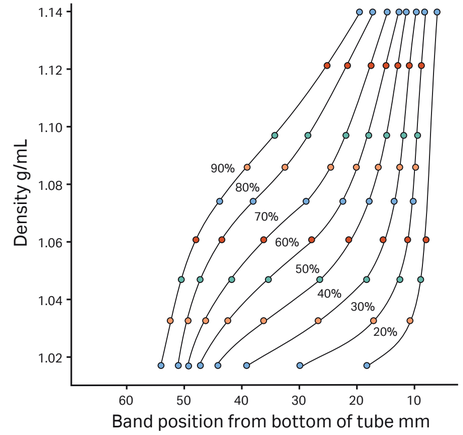


Fig 10. Use of colored Density Marker Beads to show gradient shapes. Dilutions of Percoll as in Figure 9, running conditions: 23° angle-head rotor, 60 000 × g, 15 min. Steeper gradients were formed by the greater g-force (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

Discontinuous (step) gradients

Discontinuous gradients offer great flexibility and ease of use. Often, only a cushion of Percoll or a single step is all that is required to achieve excellent enrichment or resolution of a target cell type. For example, most blood cells can be enriched using discontinuous gradients (66,69) (Fig 11).

To form a discontinuous gradient, SIP is diluted to a series of different densities as described on page 10. The solutions of different density are then carefully layered in order of density one on top of another, starting with the most dense at the bottom of the tube. This is most conveniently done using a pipette or a syringe fitted with a wide-bore needle. It is important to keep the tip of the instrument against the wall of the tube just above the surface of the liquid to avoid a "splash" and mixing at the interface. Formation of a sharp band of cells at a interface will occur only if there is a sharp change in density.

Centrifugation is performed using relatively gentle condition, such as 400 × g for 15 to 20 min in a bench-top centrifuge. These gentle conditions result in the isopycnic banding of cells at the relevant interfaces. The low-g conditions and short run time will not cause sedimentation of the Percoll and will not affect the gradient in any way.

Continuous linear and non-linear gradients

Continuous gradients are characterized by a smooth change in density from the top to the bottom of the tube. Instead of the obvious interfaces present in the discontinuous gradient, a continuous gradient can be thought of as having an infinitive number of interfaces. Therefore, isopycnic banding of cells occurs at the precise density of the cell.

To form such a gradient, SIP is first diluted to produce two solutions of known density at the limits of the range required, and then mixed using a dual-chamber gradient maker (e.g., Cytiva Gradient Mixer GM-1). A linear gradient spanning the range between the limits of the two starting solutions is formed.

A single-channel peristaltic pump (e.g., Cytiva Peristaltic Pump P-1) in combination with a gradient mixer can be used to generate linear, convex, and concave gradients, depending upon the relative diameters of the tubing used. A very narrow range of densities from top to bottom of the gradient can be formed to effect a maximum resolution of viable cells. Heavier cells usually pellet, while non-viable cells are found at the top of the gradient. For example, erythrocytes will pellet if the density at the bottom of the gradient does not exceed 1.08 g/mL. Density Marker Beads can be used as an external marker in a tube containing an identical gradient to that in the sample tube.

The centrifugation conditions necessary to achieve a separation are the same as those for the discontinuous gradients. Examples of separations performed on continuous gradients include the purification of Leydig cells (31), lactotrophs (19), bone marrow cells (52), intestinal epithelial cells (18), marine microalgae (28, 60) and chloroplasts (49, 58, 76, 88, 109).

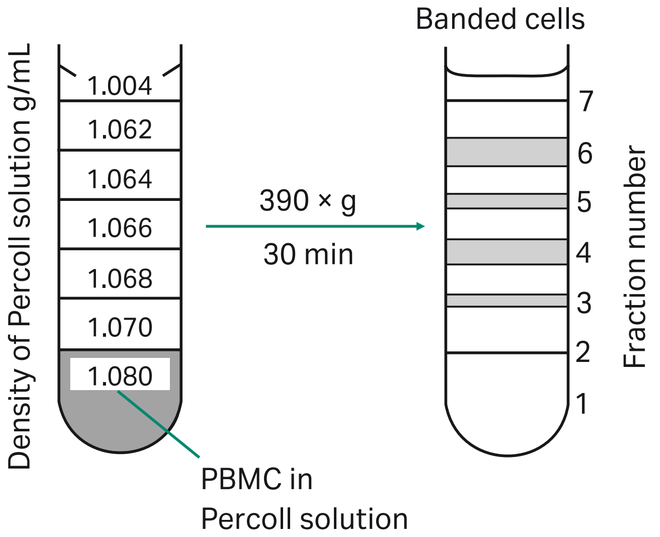


Fig 11. Separation of lymphocytes and monocytes by discontinuous density centrifugation in Percoll. 1.5 to 2.0 × 10^7 PBMC (peripheral blood mononuclear cells) isolated on Ficoll-Paque™ were mixed in 11.25 mL of Percoll in Hanks BSS containing 1% HEPES buffer (density = 1.080 g/mL) and underlayered below the steps shown in the figure (69, reproduced by kind permission of the authors and publisher).

Preformed self-generated gradients

Preforming a gradient by centrifugation can be a convenient alternative to using a gradient maker or pump. As described earlier, Percoll will sediment when subjected to significant g-forces (i.e., > 10 000 × g). When preforming a gradient, SIP is diluted to a density that lies in the middle of the range in which maximum resolution is required. Two centrifuge tubes are filled with gradient material (one for the experiment and one containing Density Marker Beads). This second tube serves both as a counter-balance and as an external method for monitoring the gradient. The tubes are centrifuged in an angle-head rotor (e.g., 30 000 × g for 15 min), and the gradient forms isometrically around the starting density (Fig 4). The relatively "flat" region of the gradient should encompass the range required for maximum resolution of the target cells. This can be confirmed by observing the shape of the gradient in the tube containing the Density Marker Beads. The gradient becomes progressively steeper with time. It has been shown that the shape of the gradient is approximately linear related to the total g-force and time of the centrifugation (22).

After forming the gradient, isopycnic banding of cells can be accomplished by low-speed centrifugation for 15 to 20 min at 400 × g. If an estimate of cell density is required, a volume equal to that of the cell suspension is layered on top of the tube containing the Density Marker Beads. This serves as both a way to estimate cell density and as a counter-balance.

Gradients formed *in situ*

The sedimentation coefficients of subcellular particles and viruses are usually too low to allow banding on preformed gradients at low g-forces. Therefore, it is often convenient to mix the suspension of biological particles with Percoll and to band the particles on a gradient formed in situ. Gradients of Percoll formed by centrifugation are metastable (i.e., they will change continuously during high speed centrifugation). The rate of sedimentation of the colloid is slow enough to allow the banding of small viruses and cell organelles with "S" values > 60S as the gradient is formed *in situ*.

A common method for forming gradients *in situ* is to prepare a SIP, using 9 parts of Percoll to 1 part of 2.5 M sucrose. The SIP is then diluted to the desired density using 0.25 M sucrose. (Although sucrose is typically used to make *in situ* gradients, cell culture media can also be used). When mixing the sample directly with gradient material, the effect on the overall density of the Percoll solution can be calculated from the formula on page 11. Premixing of the sample with the gradient material is convenient when it is desirable to accurately measure the buoyant density of the particles. However, it may be better to layer the experimental sample on top of the gradient material, particularly in cases where it is desirable to separate subcellular particles from soluble proteins. The soluble proteins will remain in the buffer layer above the gradient and subcellular particles will separate in the Percoll gradient *in situ*.

Centrifugation must be carried out in an angle-head rotor. A balance tube containing Density Marker Beads in place of experimental sample is used to monitor the gradient. An appropriate model experiment similar to the one described on page 21, should be carried out first to establish the gradient formation characteristics of the rotor to be used.

Maximum sample loading

There are no standard rules governing the maximum quantity of cells or subcellular material which can be separated on gradients of Percoll. For subcellular fractionation, successful purification can be achieved with a total loading of 1 to 5 mg of protein in a samlpe volume of 0.5 mL on 10 mL of gradient material (Pertoft, personal communication).

A model experiment to standardize conditions

The exact shape and range of gradients formed during centrifugation is influenced by the model and angle of the rotor used, and by the size of the centrifuge tubes. The following experiment is designed to enable you to establish a series of gradient curves for a particular rotor and tubes, and can be used as a reference for all future experiments.

The experiment can be repeated using Percoll in 0.25 M sucrose; in this case, running conditions should be 50 000 × g for 25 min followed by 100 000 × g for 25 min.

The example chosen is for 10 mL gradients, but this may be scaled up for larger tube sizes.

1. Mix 49.5 mL of Percoll with 5.5 mL of 1.5 M NaCl to make a SIP.
2. Mix SIP from step 1 with 0.15 M NaCl to make a series of 10 mL experimental samples (total centrifuge tube size = 13.5 mL) as shown in the following table:

Tube No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Percoll (SIP) (mL)	10	9	8	7	6	5	4	3	2	1
0.15 M NaCl (mL)	-	1	2	3	4	5	6	7	8	9

3. Add 10 µl of a suspension of each type of Density Marker Beads to each tube according to the instructions supplied in the pack.
4. Balance and cap the tubes, and mix them by inverting several times.
5. Place the tubes in the angle-head rotor (if there are only 8 spaces, omit tubes 1 and 10).
6. Centrifuge at 30 000 × g for 15 min.
7. Carefully remove the tubes and using millimeter graph paper, measure to the nearest 0.5 mm the distance of each band from the bottom of the tube.
8. Plot the gradient shape for each tube by calibrating each band with the exact buoyant density for each Marker Bead.
9. Re-mix the contents of each tube by inversion and repeat the centrifugation, this time using 60 000 × g for 15 min.
10. Measure the gradients and plot the results as before. Calculate the exact density of the dilution using the formula (see page 11). Figures 9 and 10 show typical examples of a series of curves generated using Percoll in 0.15 M NaCl.

How to fractionate and analyze gradients of Percoll

Density determination using Density Marker Beads

Using Density Marker Beads as an external marker facilitates monitoring of the gradient shape and range. The position of cells or organelles within the gradient may be accurately located before fractionation using preformed gradients (73, 83). The densities of the Density Marker Beads cover the buoyant densities of the vast majority of cells and organelles to be separated in Percoll. In addition to providing a very rapid and simple method for density measurement, using Density Marker Beads provides more accurate data than other methods, since distortion of gradients by fractionation before analysis is completely avoided.

Density Marker Beads are also very useful for standardizing running conditions before carrying out an actual experiment, using the model experiment described previously to generate a series of gradient curves specific for a particular rotor and tube type.

Density Marker Bead — properties

Each vial contains freeze-dried cross-linked dextran beads having an accurately determined density in Percoll. Nine of the ten bead types can be used for gradients of Percoll containing 0.15 M NaCl or 0.25 M sucrose. Vial 5 is used exclusively for Percoll with 0.15 M NaCl and vial 10 contains beads to be used only for Percoll with 0.25 M sucrose.

Volume of beads swollen in water:	0.7 mL/vial
Density of each bead type:	calibrated to ± 0.0005 g/mL
Total density range covered:	1.017–1.142 g/mL for Percoll in 0.15 M NaCl 1.037–1.136 g/mL for Percoll in 0.25 M sucrose

For detailed information on the properties and use on Density Marker Beads please refer to manufacturer's technical information and instructions for use.

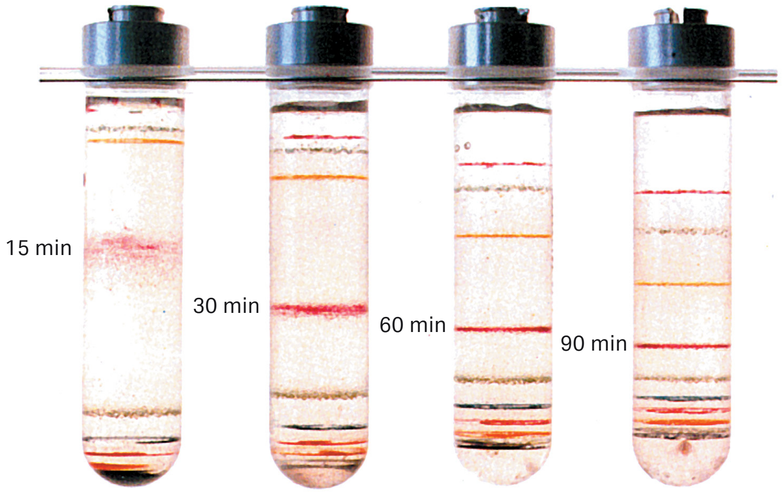


Fig 12. Banding of Density Marker Beads in gradients of Percoll as described in Figure 4 (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

Effects of ionic strength and sucrose concentration on density of Density Marker Beads

The actual buoyant density of the beads will vary slightly with ionic strength or sucrose concentration (osmolality). Figure 13 shows variations of density with ionic strength and Figure 14 shows variations with sucrose concentration. When working with systems outside the normal range of ionic strength or osmolality, these figures may be used as a guideline for calibration of bead densities.

Figure 15 shows the correlation of densities calibrated with Density Marker Beads and by a digital densitometer. This latter method may be used as a crosscheck when working with Percoll in systems outside normal physiological conditions.

Using Density Marker Beads

The beads must be swollen with water prior to use; 1.0 mL of sterile water is added to each vial and the beads are allowed to swell overnight. For long term storage of beads in water, it is advisable to add a preservative such as Merthiolate™ (0.01% w/v).

The quantity of beads required for each experiment will depend on the size of the centrifuge tube, but 10 to 15 µl of suspension is usually sufficient for 10 mL of Percoll. When dispensing the beads with a micropipette, it is useful to snip off the end of the disposable plastic tip to avoid clogging by the beads.

The size of the Density Marker Beads is sufficiently small for them to pass through tubing, monitoring equipment, etc., without problems. Density Marker Beads have been used to monitor gradients of Percoll in zonal centrifuge rotors.

Density Marker Beads are used as external markers, in a centrifuge tube containing identical gradient material to the one used for the experiment. They should not be mixed with the cell sample. Density Marker Beads are added to the control tube, which is then used as a counter-balance in the rotor during the centrifugation. The shape of the gradient is measured as described in the model experiment on page 21.

For detailed information on the properties and use on Density Marker Beads please refer to the manufacturer's technical information and instructions for use.

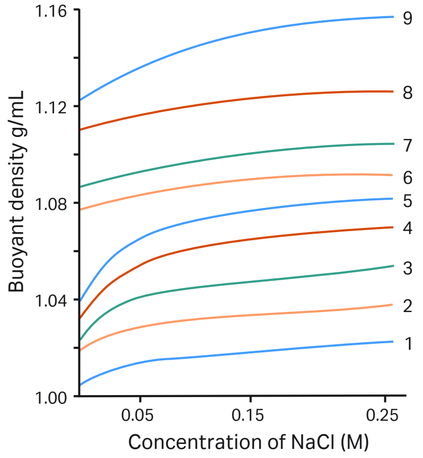


Fig 13. Effects of salt concentration on the recorded densities of Density Marker Beads in gradients of Percoll. Numbers refer to different bead types (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

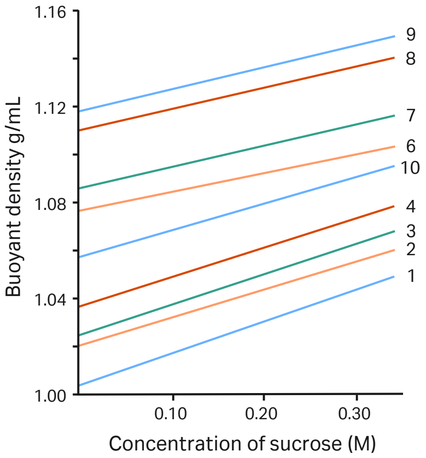


Fig 14. Effects of sucrose concentration on the recorded densities of Density Marker Beads in gradients of Percoll. Numbers refer to different bead types (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

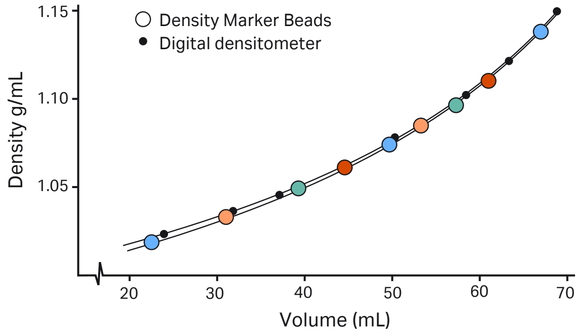


Fig 15. Correlation of recorded densities of a Percoll gradient in 0.15 M NaCl using Density Marker Beads and a digital densitometer (DMA 46, Anton Paar A.G.). Fraction size 2.64 mL, centrifuge MSE Superspeed 75, rotor 10 × 100 mL, angle 18°, 40 000 × g for 60 min (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

Other methods for measuring density

Several techniques can be used to monitor the density of Percoll solutions after fractionation. Weighing of empty and filled glass micropipettes is accurate but tedious. It is also possible to measure the isopycnic equilibrium point of samples in a precalibrated gradient made from nonaqueous organic liquids (12). Refractive index has a linear correlation with the density of a Percoll solution as shown in Figure 16. Direct measurement using a densitometer (e.g., DMA 3, Anton Paar A.G.) is an accurate alternative to using Density Marker Beads (Fig 15).

Fractionation of gradients

After centrifugation, the gradient can be fractionated by puncturing the bottom of the tube and collecting the outflow into fractions, or by a number of other techniques (1, 28). A simple and convenient method is to collect the fractions from the top of the tube by displacement with a dense medium such as undiluted Percoll, or a 60 to 65% sucrose solution. Upon pumping this dense material to the bottom of the tube, fractions can be drawn off the top. Zonal rotors may be emptied by pumping a denser solution to the distal part of the rotor and collecting fractions from the center.

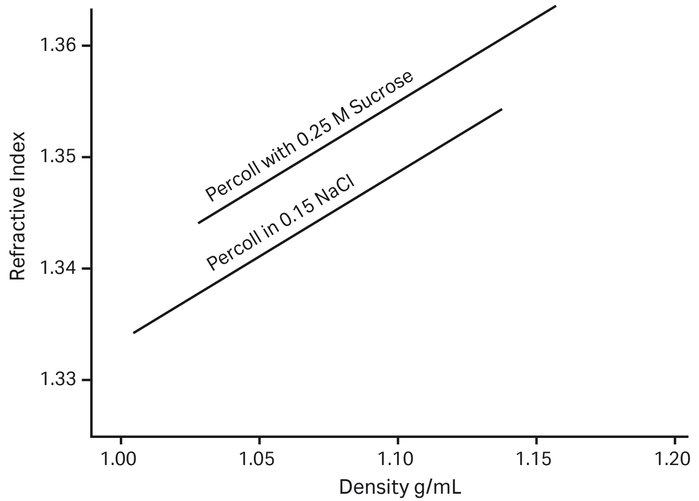


Fig 16. Refractive index as a function of density of a Percoll gradient (work from Cytiva Bio-Sciences AB, Uppsala, Sweden).

Cell sorting and counting

Percoll does not interfere with fluorescent activated cell sorting (FACS) (911, 1042), or with electronic counting instruments (12). The DNA content of gradient fractions can also be used as a measurement of cell number (12).

Protein determination and enzyme assay

Percoll causes a background color with Folin-Ciocalteau and Lowry reagents, and all measurements should use Percoll solutions for the preparation of the blank. Higher protein concentrations can be determined using the biuret reaction (85). Terland *et al.* (89) recommend the Coomassie™ Blue method of Bradford (90), since Percoll does not interfere with color development. Vincent and Nadeau (518) have reported a modification of Bradford´s method which involves precipitation of Percoll in a NaOH Triton™ X-100 mixture.

Cell organelles are often identified primarily by the presence of specific enzymes. Many enzyme assays can be carried out in the presence of Percoll without interference. Pertoft and Laurent (21) described an experiment in which the enzymes 5′-nucleotidase (plasma membranes), glucose-6-phosphatase (microsomes), β-glucuronidase (lysosomes) and succinic dehydrogenase (mitochondria) from rat liver homogenates were analyzed in the presence of Percoll. In all cases, the activities were at least as high in Percoll as in the controls indicating that the determinations were not influenced by the medium. Labile succinic dehydrogenase activity was stabilized by Percoll. Aryl sulphatase, alkaline phosphatase, acid phosphatase, β-galactosidase, N-acetyl-α-D-glucosaminidase and β-glucosaminidase have also been analyzed in the presence of Percoll without interference from the medium (21). Due to light scattering by Percoll, it is preferable to use enzyme assays which utilize fluorescence rather than absorbance for detection of activity. For further details of enzyme measurements in Percoll, see references 13, 43, 53, 54, 78 and 89.

Removal of Percoll after centrifugation

Since Percoll is non-toxic to biological materials and does not adhere to membranes, it is usually unnecessary to remove Percoll from the purified preparation. Cells can be transferred directly to cell culture systems (23, 57), virus infectivity is unimpaired (21), and organelles can be used for metabolic studies (21) without any effect caused by the gradient material.

The following methods can be used to eliminate the gradient material if desirable.

Washing (low speed centrifugation)

Living cells can be separated from Percoll medium by washing with physiological saline (5 volumes saline to 1 volume of Percoll cell suspension). The washing may be repeated two or three times and the cell collected between each washing step by centrifugation at 200 × g for 2 to 10 min. Studies with radioactively labeled Percoll (Table 2) have shown that no detectable residual Percoll is left adhering to cells washed in this way. Electron micrographs by Enerbäck *et al.* (9) (Fig 17) and Schumacher *et al.* (31) show cell preparations with no visible contaminating particles from the gradient material.

Washing (high speed centrifugation)

For viruses and subcellular particles which are too small to be pelleted by low speed centrifugation as described above, the biological material can be separated from coated silica particles by high speed centrifugation in a swinging bucket rotor or angle-head rotor. The undiluted fraction obtained from the first centrifugation run is placed in a centrifuge tube and spun in a swinging bucket rotor at 100 000 × g for 2 h, or 90 min in an angle-head rotor (100 000 × g) to pellet the Percoll. The biological material remains above the hard pellet of Percoll (12, 39).

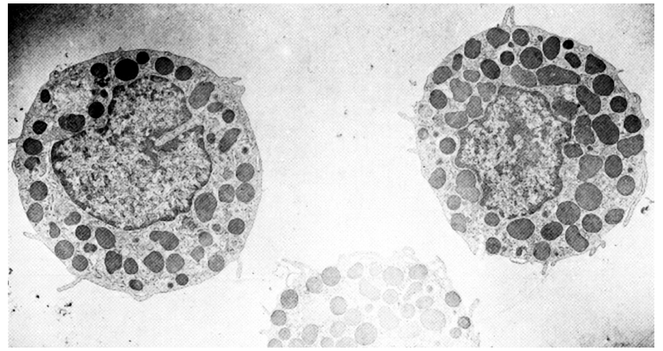


Fig 17. Electron micrograph of mast cells isolated by gradient centrifugation on Percoll (9, reproduced by kind permission of the authors and publisher).

Table 2. Removal of Percoll from rat liver hepatocytes

^{125}I-labeled Percoll was used to isolate hepatocytes in Eagle's MEM at a density of 1.07 to 1.09 g/mL.

	^{125}I (cpm)
5 mL of the original cell suspension in Percoll	35 680
Cell pellet (from 5 mL of the original cell suspension in Percoll) washed with 80 mL of Eagle's MEM and centrifuged at 200 × g for 10 min	71
Washing repeated once	0
Cells from 2 mL of the cell suspension were seeded on a 6 cm Petri dish and 80% of the cells attached to the dish. After four washings with 5 mL portions of Eagle's MEM, the cells were detached with 0.01% trypsin plus 0.25% EDTA.	0

(Original work by Pertoft *et al.* reproduced by kind permission)

Other methods

Chromatography by gel filtration on Sephacryl™ S-1000 Superfine will separate Percoll from larger particles (e.g., subcellular particles), which are eluted in the void volume. Removal of Percoll from microsomal vesicles by gel filtration on Sephacryl S-1000 Superfine has been reported (275). The authors followed the elution pattern by assaying for the microsomal marker enzyme NADPH-cytochrome c reductase (Fig 18). The resulting microsomal fraction was examined by electron microscopy and found to be almost free from Percoll (less than 0.5% compared with the initial sample).

Preliminary experiments using electrophoresis to separate lysosomes and viruses from Percoll have been reported (21), but the methodology is difficult and results are often unpredictable (Pertoft, personal communication).

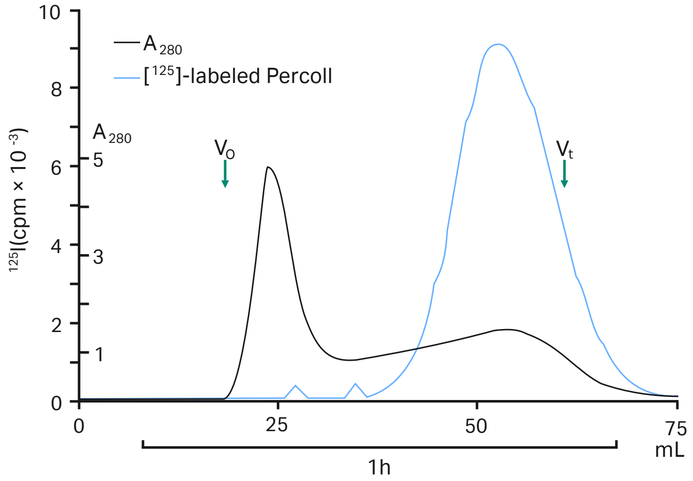


Fig 18. Gel filtration of microsomes obtained from gradients of Percoll containing ^{125}I-labeled Percoll on Sephacryl S-1000 Superfine. V_o = void volume, V_t = total volume (275, reproduced by kind permission of the authors and publisher).

Practical notes

Care and cleaning of equipment

Polycarbonate tubes should be used with Percoll as the particles do not adhere to the walls of these tubes. Solutions of Percoll usually produce a small pellet of compacted silica at the bottom of the tube after centrifugation and deposits on the wall of tubing used for fractionation etc. These deposits may be difficult to remove when dry. Therefore, it is recommended that all equipment is washed immediately after use. Spillages of Percoll can be removed by washing with water.

Storage of Percoll

Percoll can be stored unopened at room temperature for up to 5 years. When opened, it should be stored below 8°C. If opened under non-sterile conditions, Percoll may be frozen for up to 6 months at -18°C (allowing sufficient headspace for expansion) to avoid microbial growth. If stored at -18°C, gradients form upon thawing, necessitating a mixing of the contents of the bottle before use. Preformed gradients can be stored for weeks without a change in gradient shape, provided that the gradient is sterile and is not physically disturbed.

Sterilization of Percoll solutions

Autoclaving of Percoll solutions must be carried out in the absence of salts or sucrose (i.e., do not autoclave SIP). When autoclaving undiluted Percoll, it is recommended that minimum contact with air be maintained to avoid particle aggregation at the Percoll/air interface. This can be accomplished by using a narrow-necked bottle when autoclaving. If these particles form, they may be removed by low speed centrifugation. If any significant evaporation occurs during autoclaving, the volume should be replenished with sterile water so that the density is not affected.

Aggregates of silica particles

It is an inherent tendency of all silica colloids to form aggregates, either during autoclaving as described above, or upon prolonged storage. These aggregates may be observed in some batches of Percoll either as a slight precipitated sediment or as a faint white band which has a density of 1.04 to 1.05 g/mL. This band may form during gradient formation in the centrifuge or during low speed centrifugation of a preformed gradient. The aggregated silica does not interfere with the separation of biological particles as almost all cells and organelles have buoyant densities in Percoll of greater than 1.05 g/mL.

Percoll PLUS — low endotoxin

Physical properties

Composition	Colloidal silica solution with covalently linked silane	pH	9.4 ± 0.5 at 20°C
Osmolality	< 30 mOsm/kg H_2O	Carbon content in dry residue	4.0 to 5.5%
Density	1.130 ± 0.005 g/mL	Endotoxin activity (max)	2 EU/mL
Viscosity	< 15 cP at 20°C	Shelf life	5 y

Composition

Percoll PLUS is a silica-based colloidal medium for cell preparation by density gradient centrifugation. It provides all the advantages of Percoll and can be incorporated into existing procedures using Percoll gradients for the preparation of a variety of human cell types. The silica particles of the medium are covalently coated with silane providing greater product stability and low osmolality, toxicity, and viscosity.

Osmolality

Percoll PLUS has a low osmolality of < 30 mOsm/kg H_2O and can easily be adjusted with physiological saline, other balanced salt solutions, or cell culture media to give gradients that are iso-osmotic and adjusted to physiological conditions throughout.

Density

Percoll PLUS is provided having a density of 1.130 ± 0.005 g/mL. After adjustment, Percoll PLUS forms iso-osmotic gradients within the density range of 1.0 to 1.3 g/mL. This density range is optimized for separation of most cells, subcellular particles, and larger viruses, which have a buoyant density of 1.0 to 1.2 g/mL in Percoll PLUS.

Endotoxin activity

The Percoll PLUS medium has low endotoxin levels (< 2 EU/mL). Low toxicity improves safety making Percoll PLUS well-suited for cell separation in clinical research applications.

Gradients

Under moderate centrifugal force, the colloidal particles in Percoll PLUS medium sediment to form smooth, continuous density gradients and this property can be exploited in either fixed-angle or vertical rotors.

Percoll PLUS is also ideally suited to applications where high-speed centrifugation is required. In this case, the sample can be pre-mixed with the medium and subsequently separated on the continuous gradient formed *in situ*. Thus, gradient formation and sample separation can be achieved in one step.

Further details on centrifugation conditions and buoyant densities of cells, subcellular particles, and viruses centrifuged on Percoll gradients can be found elsewhere in this handbook.

Practical notes

Storage

See page 28, *Practical notes* for Percoll.

Applications

Blood cells

The entire spectrum of cell types present in blood can be resolved on preformed gradients of Percoll. The method described by Pertoft *et al*. (55) (Fig 19) utilizes both rate zonal (separation by size) and isopycnic (separation by density) techniques. Diluted blood was layered on top of a preformed self-generated gradient and centrifuged for 5 min at 400 × g, during which time the thrombocytes or platelets (which are appreciably smaller than the other cells present) did not penetrate into the gradient.

The plasma layer containing the thrombocytes was removed and replaced by saline, and centrifugation was continued at 800 × g for 15 min, resulting in isopycnic banding of mononuclear cells (lymphocytes and monocytes), polymorphonuclear cells and erythrocytes. The position and densities of the banded cells were monitored using Density Marker Beads in an identical gradient contained in a second centrifuge tube.

Although the above method demonstrates the utility of Percoll for fractionating whole blood, most blood cells can be appreciably enriched using a simple step gradient. A simple step gradient often gives acceptable yields and purity for downstream processing. The following Application tables contain a number of examples of purification of blood cells and other cell types using different types of Percoll gradients.

The following tables were complied to assist the researcher in selecting references most likely to contain relevant information regarding use of Percoll for a particular cell or tissue type.

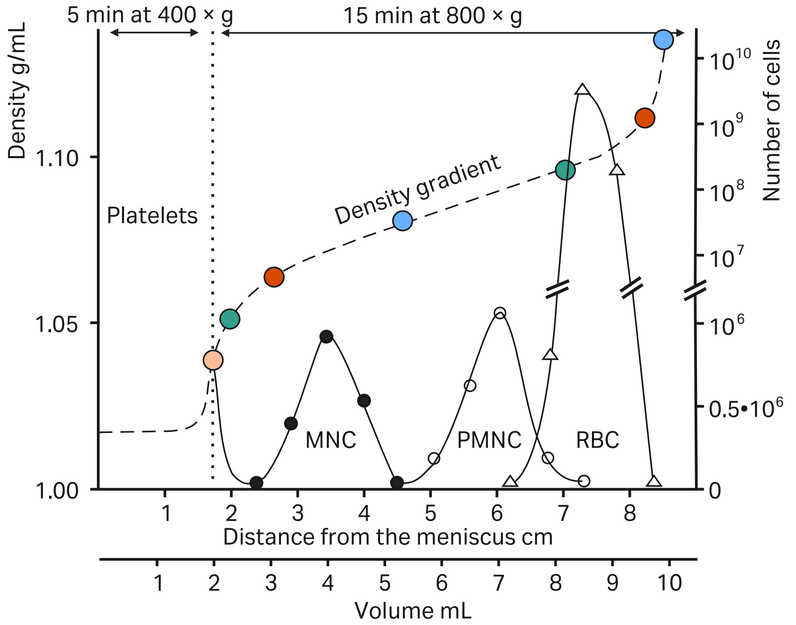


Fig 19. Separation of human blood cells in a gradient of Percoll. The tubes were filled with 10 mL of 70% (v/v) Percoll in 0.15 M NaCl (p=1.086 g/mL), and the gradient performed by spinning in a 14° angle rotor at 20 000 × g for 15 min. Two mL of gradient material was removed from the bottom of the tube using a syringe, and 1 mL of heparinized blood diluted with 1 mL of 0.15 M NaCl was layered on top of the gradient. Centrifugation was carried out as indicated. Densities were monitored using Density Marker Beads. MNC = Mononuclear cells, PMNC = Polymorphonuclear cells, RBC = Red blood cells (55, reproduced by kind permission of the authors and publisher).

Applications — blood cells

Lymphocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	blood	Percoll density centrifugation resulted in significant down-regulation of L-selectin surface reactivity.	Immunoflourescence	891
human	continuous	tonsil	A Percoll density gradient was used for separation of large (low density) *in vivo* activated cells from small (high density) resting cells.	cell culture, FACS, granulocyte-macrophage colony stimulating factor (GM-CSF) assays, and Northern blots	892
human	continuous	spleen, tonsil	Large B lymphocytes from tonsils (*in vivo* activated cells) obtained by Percoll gradient centrifugation displayed higher IL-4R levels than resting cells.	cell culture, Northern blots, FACS	893
human	continuous	peripheral blood	Percoll was used to separate proliferating form nonproliferating cells.	tritiated thymidine incorporation	11
human	continuous	tonsil, peripheral blood	This procedure yielded > 90% viable cells and has proved quite helpful in renewing overgrown cultures.	proliferation and cytotoxicity assays	16
human	continuous	blood	Percoll was used to separate monocytes from lymphocytes.	cell culture, coagulation activity, immunoradiometric assays	40
human	discontinuous (3-layer)	intestine	Lymphocytes were enriched in the interface between 66.7 and 44% Percoll. Further purification was performed using magnetic beads.	flow cytometric analysis, immunoperoxidase procedure, cell culture	894
human	discontinuous (6-layer)	peripheral blood	Percoll was used to separate large granular lymphocytes (LGL) from peripheral mononuclear cells.	detection of $CD5^{LOW+}$ in the LGL population	895
human	discontinuous	tonsil	Percoll gradient was used for the separation of small (high density) and large (low density) cells.	cell culture, apoptosis assays, immunoassay for G-CSF, bioassay for GM-CSF, Northern blot analysis	896
human	discontinuous	intestine		proliferation assays, measurement of cytotoxicity, H1 receptor binding studies	897
human	discontinuous	peripheral blood	Percoll was used for the isolation of low density cells.	FACS, immunoflourescence, nonspecific esterase staining	898
human	discontinuous	peripheral blood	Lymphoctes were recovered from low density Percoll fractions.	suppression of NK-cell proliferation by freshly isolated monocytes	899
human	discontinuous	tonsil	Percoll was used to isolate follicular dendrite cells (FDCs).	cell sorting, B cell proliferation by FDCs	900
human	discontinuous	bone marrow	Percoll was used to isolate leukemic cells from bone marrow.	establishment of a leukemic cell line	901

Lymphocytes (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	peripheral blood	After separation on Percoll, a virtually pure population of activated cells was obtained, as estimated by the presence of the 4F2 marker and of the transferrin receptor.	immunoflourescence and assay of phospholipid metabolism	902
human	discontinuous (1-step)	blood	Lymphocyte purity was > 99% and the population of monocytes was enriched 82 to 90%.	induction and assay of lymphokine (IL-2)-activated killer (LAK) cell activity	903
human	discontinuous (4-layer)	blood	Percoll was used for separation of large granular lymphocytic (LGL) cells from T cells.	Giemsa staining, cell activation with interleukin-2 (IL-2)	904
human	discontinuous (4-layer)	tonsil	Percoll was used for B cell enrichment.	flow cytometry	905
human	discontinuous (5-layer)	blood	Large granular lymphocytes (LGL) were collected from the low density fractions, whereas T cells were located in the higher density bottom fraction.	FACS, cell culture, cytotoxicity assays	906
human	discontinuous (5-layer)	blood	Monocytes were purified up to 90% and lymphocytes to > 99%.	cell counting (hemocytometer) and cell culture assays	69
human	discontinuous (7-layer)	peripheral blood		cytotoxicity assay, flow cytometry analysis, and complement-dependent lysis	907
human	self-generating	peripheral blood	Percoll was used to separate viable and nonviable cells. Yields were slightly higher and erythrocyte contamination was slightly lower with Percoll than with Ficoll-Isopaque.	cytotoxicity assays	83
canine	continuous	blood	Percoll was used for enrichment and depletion of antibody-positive cells.	reverse hemolytic plaque assay and cell-mediated lympholysis	908
canine	discontinuous (4-step and 2-layer)	whole blood	A final sedimentation of purified lymphocytes through a 45/50% Percoll gradient concentrated natural killer (NK) activity into a single band of lymphocytes.	measurement of NK activity	909
mouse	continuous (3-layer)	intestine	Enrichment increased from 44.1% (single filtration) to 52.4% (multiple filtration) after nylon wool filtration, and from 70.3% (single filtration) to 82.8% (multiple filtration) after Percoll fraction.	flow cytometry	910
mouse	continuous (5-layer)	spleen	Percoll was used for separation of virgin and memory T cells.	cell proliferation assays, FACS	911
mouse	discontinuous (3-layer)	spleen	Percoll was used for separation of B cells.	protein phosphorylation assay	912
mouse	discontinuous (3-layer)	intestine	Percoll was used for isolation of intestinal intraepithelial lymphocytes (IEL).	DNA analysis by flow cytometry, mRNA-cDNA dot blots, PCR	913
mouse	discontinuous (4-layer)	spleen	Percoll was used for isolation of small, resting B cells.	cell cycle analysis by flow cytometry	914
bovine	discontinuous	mammary	Purified cells were > 80% pure.	Wright´s Giemsa staining, cell culture	915

Monocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (minigradient)	peripheral blood	With the Percoll minigradient, cells could be obtained in 90 to 100% from the patients at all time points after bone marrow transplant (BMT).	cytogenic analysis	916
human	continuous	blood	The isolated mononuclear leukocyte (MNL) fraction contained > 80% cells giving a positive reaction for α-naphthyl acetate esterase (α-NAE).	cell culture	917
human	continuous	peripheral blood	Percoll was used to isolate monocytes with > 85% purity and > 95% viability.	cell culture with cytokines	918
human	continuous	blood	Percoll has proved very practical for the separation of monocytes from blood and of macrophages from ascites and synovial fluids.	cell culture	34
human	continuous	blood	Percoll gradients were used for the separation of monocytes from lymphocytes.	cell culture	40
human	continuous	blood	A one-step procedure was used for obtaining a high-yield suspension of monocytes of 20% purity, which does not require washing before cultivation. A two-step method gave better than 90% pure monocytes at a lower yield.	cell counts, Fc-receptor presence and phagocytosis assays	57
human	continuous	peripheral blood	MNL were separated into two fractions with Percoll: one consisting mostly of monocytes and the other lymphocytes.	fungal (*Coccidioides immits*) killing assay	919
human	discontinuous	blood	Monocyte purity was 95%.	cell culture	920
human	discontinuous	whole blood	Percoll gradient was used for enrichment of hematopoietic progenitor cells.	assay for colony formation	921
human	discontinuous	blood		RNA isolation, Northern blot analysis and RT-PCR	922
human	discontinuous	bone marrow		DNA hybridization studies	923
human	discontinuous (1-layer)	blood	Lymphocyte purity was > 99% and the population of monocytes was enriched 82 to 90%.	induction and assay of lymphokine (IL-2)-activated killer (LAK) activity	903
human	discontinuous (1-layer)	blood	PMN recovery was > 90% and RBC contamination < 5%.	Northern blot analysis	924
human	discontinuous (1-layer)	peripheral blood	Monocytes were ≥ 95% pure.	Northern blot analysis, nuclear runoff experiments, S1 protection assay	925
human	discontinuous (4-layer)	peripheral blood	Cells obtained from the 65% to 75% interface were 99% granulocytes.	analysis and Western blot analysis genomic DNA isolation and PCR	926
human	discontinuous (1-layer)	peripheral blood	With the 1-step gradient, the purity of the monocytes was 93 to 96%.	Giemsa staining and cell culture	927

Monocytes (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (5-layer)	peripheral blood	Percoll-isolated monocyte/macrophages as identified by Wright-Giemsa stain.	interactions between monocyte/macrophage and vascular smooth muscle cells	928
human	discontinuous (5-layer)	blood	Monocytes were purified up to 90% and lymphocytes to 99% purity.	cell recovery counting and cell culture assays	69
human	discontinuous	peripheral blood		cell enumeration with Coulter counter, RNA isolation, and Northern blot analysis	929
equine	discontinuous (1-layer)	peripheral blood	All MNCs were recovered on Percoll gradients without any neutrophil contamination.	cell recovery assays	930

Erythrocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	whole blood	Percoll was used for separating young and old erythrocytes.	immunoflourescence analysis of complement receptor type 1 (CR1) and CD59, proteolytic cleavage of CR1 *in vivo*	931
human	continuous	blood	Percoll was used to separate *Plasmodium falciparum*-parasitized erythrocytes from nonparasitized erythrocytes.	isolation of erythrocyte membranes lipid peroxidation, vitamin E and transmembrane reducing system analysis	932
human	continuous	blood	A rapid method for the age fractionation of human erythrocytes by Percoll density gradient centrifugation was described.	flame photometry, enzyme assays	77
human	discontinuous (4-layer and 8-layer)	blood	A rapid method using Percoll to fractionate erythrocytes according to age was described.	analysis of the decline of enzymatic activity in aging erythrocytes	933
human	discontinuous (4-layer)	blood		ELISAs, proteolytic digestion of membranes	934
human	discontinuous (4-layer)	blood	The position of Density Marker Beads was used to collect cells with densities < 1.00 g/cm^3 or > 1.119 g/cm^3.	yield stress experiment: a sensitive index of cell: cell adhesion of deoxygenated suspensions of sickle cells	935
human	discontinuous	blood	Percoll gradient was used to separate erythrocytes into 4 density fractions.	platelet-activating factor (PAF) acetylhydrolase activity and membrane fluidity	936

Erythrocytes (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	blood	Erythrocytes loaded with L-asparaginase using a hypotonic dialysis process were separated into eight fractions.	L-asparaginase activity	937
human	discontinuous	blood	Discontinuous gradient of the range 1.080 to 1.115 g/cm^3 with each layer differing in density by 0.005 g/mL produced nine cell fractions.	enzyme assays	66
human	discontinuous (5-layer)	blood		study of RBC deformability and cell age	938
human	discontinuous (8-layer)	blood	Percoll was used for density separation of RBC loaded with inositol hexaphosphate (IHP) by reverse osmotic lysis.	haemoglobin distribution, distribution of IHP concentrations	939
human	discontinuous (9-layer)	blood	A detailed comparison between two cell-loading techniques for inositol hexaphosphate was performed by monitoring the RBC distribution patterns on Percoll density gradients.	oxygen affinity, hematological parameters and organic phosphate content measurements	940
Mastomys natalensis	continuous	blood	Percoll was used to separate *Plasmodium berghei*-parasitized erythrocytes from non parasitized cells.	cAMP level in RBCs	941
mouse	continuous (self-forming)	blood	Fractionation of RBC yielded five distinct populations that maintained their densities upon recentrifugation in a second gradient.	transbilateral movement and equilibrium distribution of lipid	942
mouse	continuous	peripheral blood	Percoll was used for density gradient separation of chemically-induced erythrocytes.	fixing, staining and flow cytometric analysis of micronucleated polychromatic (MPCE) and micronucleated nonchromatic (MNCE) erythrocytes	943
mouse	discontinuous	peripheral blood	Erythrocytes were contaminated with only 0.001% nucleated cells.	glucose phosphate isomerase (GPI) assay	944
rat	discontinuous	whole blood	Percoll was used to separate Plasmodium berghei-infected RBCs.	oxygen dissociation analysis	945
rabbit	discontinuous (7-layer)	blood	Rabbit red blood cells were reproducibly fractionated into populations of various stages of maturation.	measurement of cytosolic protease activities	946
trout	discontinuous	blood	The gradient in the region of 45 to 65% Percoll produced three red cell fractions which is due to multiplicity of haemoglobin components.	antioxidant enzyme activities and membrane fluidity analysis	947

Natural Killer (NK) cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	peripheral blood	The Percoll (preculture) step facilitated the density separation of resting cells from larger lymphocytes.	NK- and T-cell activation, immunoflourescence	948
human	discontinuous	blood	K562 cells which adhere to NK cells were separated together. Enrichment of NK cells was 71.3%.	cytotoxicity studies, morphological characterization	61
human	discontinuous (2-layer)	peripheral blood	The low density fraction (42.5 to 47.5% Percoll) which showed a 4-fold enrichment in NK activity was used.	NK activity and kinetic constant determinations, measurement of the effect of divalent cations on NK activity, and effect of ATP on NK cell-surface markers	949
human	discontinuous (6-layer)	blood	Further purification using magnetic beads resulted in a pure preparation.	cytotoxic assay	950
human	discontinuous (8-layer)	peripheral blood	Recovery was > 80% while viability, as judged by trypan blue exclusion, was > 95%.	NK cell stimulatory effect, phenotype evalution by immunoflourescence	951
mouse	discontinuous (3-layer)	lung	The cells at the 50/55% interface were the richest in NK cell activity.	adoptive transfer to reconstitute NK activity in NK-depleted mice	952
mouse	discontinuous (6-layer)	spleen	NK cells were enriched in the lower density Percoll fraction, while natural cytotoxic T cells (NCT) were distributed between both higher and lower density fractions.	cytotoxicity of NK cells was measured	953
mouse	discontinuous (6-layer)	liver	All NK activity was above 1.08 g/mL density. Interfaces at 1.04 and 1.06 gave a 2× enrichment of NK progenitors.	PCR, Western blot analysis, and cytotoxicity assays	954

Neutrophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (1-layer)	whole blood	Neutrophils pelleted in the 1.077 g/mL cushion.	FACS analysis, intracellular Ca^{++} and superoxide anion measurements	955
human	discontinuous (2-layer)	whole blood		polymorphonuclear neutrophil (PMN) labeling by immunoflourescence, adherance assay and superoxide assay	956
human	discontinuous (4-layer)	peripheral blood	Percoll was used to separate monocytes and lymphocytes.	immunoflourescence and flow cytometry	957
human	discontinuous (4-layer)	whole blood	Eosinophils and neutrophils were isolated following dextran sedimentation.	flow cytometry and measurement of lactoferrin release	958
human	discontinuous	blood	Cell preparation was layered onto a Percoll cushion to remove monocytes. After lysis of the erythrocytes, primarly neutrophils, with the remaining cells being predominantly eosinophils.	immunoflourescence studies	959
human	discontinuous	blood	The neutrophils were > 95% pure.	indirect immunoflourescence, immunoelectron microscopy and FACS analysis, O_2 consumption	960
human	continuous, nonlinear (2-layer)	blood	Percoll was used for subcellular fractionation of azurophil granules, specific granules, gelatinase granules, plasma membranes, and secretory vesicles.	ELISAs for NGAL, gelatinase, lactoferrin and myeloperoxidase	961
mouse	continuous	peritoneum	An ~97% pure polymorphonuclear neutrophilic leukocyte (PMN) preparation was obtained using Percoll.	electrophoretic analysis, GM-CSF assay, and cell morphology and counts	70

Eosinophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	peripheral blood	Eosinophils were purified using Percoll gradients followed by immuno-magnetic beads. Using this procedure, the eosinophil purity was always > 95% and the viability was > 98%.	FACS analysis, eosinophil migration assays, Ca^{++} measurements	962
human	discontinuous (2-layer)	blood	The recovery of eosinophils was 40% to 60%, the viability > 98% as tested by trypan blue exclusion, and the purity > 85%.	chemotaxis and intracellular Ca^{++} measurements	963
human	discontinuous (2-layer)	blood	Eosinophil purity was > 95%, and the method did not induce priming of the eosinophils.	serum-treated Zymosan (STZ) binding and placenta-activating factor (PAF) measurments	964
human	discontinuous (2-layer)	blood	Eosinophil purity was always > 85% and the recovery ranged from 40% to 60%. Viability was > 98%.	chemotaxis assay	965
human	discontinuous (3-layer)	peripheral blood	Eosinophil purity was 95% to 99%, viability using trypan blue was > 98%, and recovery was 40% to 60%.	density distribution analysis, cell culture	966
human	discontinuous (4-layer)	whole blood	The effect of dextran sedimentation on the density of neutrophils and eosinophils was analyzed.	flow cytometry and measurement of lactroferrin release	958

Basophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	peripheral blood	Basophils were purified by Percoll density gradient separation and cell sorting. The procedure yielded 95% purity with a total yield estimated to range from 5% to 28%.	flow cytometry, histamine release, electron microscopy	967
human	continuous	bone marrow	The purity of basophils in the low density fraction (< 1.063 g/mL) was generally > 75% of the cells.	histamine content and release	968
human	discontinuous	peripheral blood	Highly purified basophils were obtained by Percoll gradient followed by negative selection using flow cytometry.	effects of cytokines on human basophil chemotaxis	969
human	discontinuous (2-layer)	blood	The majority of the basophils were located at the 1.070 to 1.080 interface. The purity in this fraction was 36% to 63%.	further purification by negative selection using immuno-magnetic beads	970
human	discontinuous (2-layer)	blood	Highly purified basophils were obtained by Percoll gradient followed by negative selection using flow cytometry.	histamine release assay, chemotactic assay	971
human	discontinuous (3-layer)	whole blood	Basophils were purified to > 80% using Percoll gradient followed by treatment with monoclonal antibodies to remove contaminants.	flow cytometry and leukotriene C4 generation following calcium ionophore stimulation	972
human	discontinuous (3-layer)	peripheral blood	Basophil purity was 85% to 96% using Percoll.	cell stimuli and mediator release assay	973
rat	discontinuous	blood		further purification by immuno-magnetic beads, immunoflourescence, electron microscopy	974

Applications — other cell types

Liver cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	liver	Purification of cryo-preserved hepatocytes on Percoll density gradients increased the percentage of viable cells from 55% to 87%.	primary cell culture, electron microscopy, viability assay radiolabeled protein synthesis, secretion assay, metabolic studies, toxicological studies	975
rat	continuous	liver	Percoll offered a good way to obtain an enriched population of Kupffer cells. Recovery was 82%, viability 87% and purity 67%.	peroxidatic reaction	20
rat	continuous	liver	Percoll gradients were used to isolate hepatocyte plasma membranes and mitochondrial membranes.	phase contrast microscopy, cell binding experiments	33
rat	continuous	liver	Rat liver cells furnished subpopulations of parenchymal cells (hepatocytes) having buoyant densities of 1.07 to 1.09 g/mL, and non-parenchymal cells (mostly phagocytosing Kupffer cells) at a density of 1.04 to 1.06 g/mL.	cell culture	55
rat	NA	liver	Final preparations contained less than 5% nonviable cells as judged by trypan blue exclusion.	cell culture	71
rat	continuous	liver	Percoll gradients were used to franctionate nonparenchymal cells into Kupffer cells, stellate and endothelial cells.	light and flourescence microscopy, carboxyesterase and Glutathione-S-transferase (GST) activities	976
rat	discontinuous (2-layer)	liver	Percoll provided a simple, low cost, and rapid method for the isolation, purification and cultivation of rat liver sinusoidal endothelial cells (LEC).	electron microscopy, cell culture, trypan blue exclusion	977
rat	discontinuous (2-step)	liver	Percoll gradients were used to separate fat storing cells (FSC) from liver endothelial cells (LEC) and Kupffer cells (KC).	cell culture	978
rat	continuous	liver	Following the removal of damaged cells by centrifugation in Percoll, the mean viability of cryo-preserved hepatocytes, tested by trypan blue exclusion, was 88.6% (±1.3%).	cell viability and study of xenobiotic metabolism	979
rat	continuous	liver	Percoll was used to remove dead cells from cryopreserved cells. Cell viability was 88 ±1% after the Percoll step.	cell viability and study of xenobiotic metabolism	980

Liver cells (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
rat	continuous	liver	If cryo-preserved cells were purified by a Percoll centrifugation after thawing, the enzyme activities were not significantly different from those of freshly isolated parenchymal cells, and the viability was 86%.	Lowry protein assay, cytochrome assay, enzyme assays	981
rat	continuous	liver	Percoll separation yielded cryo-preserved cells with a viability and metabolic capacity not measurably different from freshly isolated cells.	protein determination, enzyme assays and metabolism of testosterone and benzo(a) pyrene (BaP)	982
rat	discontinuous (2-layer)	liver	Percoll two-step gradients were used to separate Kupffer cells (KC) and liver endothelial cells (LEC). Preparations of KC were 85% to 92% homogenous while the LEC preparation was at least 95% pure.	light microscopy, electron microscopy and peroxidase staining	983
rat	discontinuous (5-layer)	liver, spleen	Percoll gradients were used to separate both spleen and liver cells. Spleen and liver cell viability was over 95%.	trypan blue viability assay, cell culture	984
rat	continuous	liver biopsy	Percoll was used for separation of hepatocytes and non-parenchymal cells, as well as subfractionation.	cell enumeration using Coulter counter, immunocytochemistry, DNA extraction, Southern blot analysis, assay of marker enzymes and protein in subcellular fractions, electron microscopy	985

Leydig cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	testis	Percoll-purified Leydig cells were 70% to 80% pure based on staining for 3 beta-hydroxysteroid dehydrogenase.	cell culture, stimulation of testosterone production	986
human	continuous	testis	Percoll-purified Leydig cells were 80% to 90% pure as determined by 3 beta-hydroxysteroid dehydrogenase staining.	immunocytochemical localization of apolipoprotein E (apoE)	987
human	discontinuous (4-layer)	testis	Percoll gradients were used to isolate human Leydig cell mesenchymal precursors.	cell culture	988
human	discontinuous (5-layer)	testis	Percoll gradient centrifugation permitted isolation of two Leydig cell fractions.	cell culture	989
mouse	continuous (linear)	testis	Two groups were obtained: group 1 had densities of 1.0667 to 1.0515 g/mL; group 2 had densities of 1.0514 to 1.0366 g/mL.	*in vitro* testosterone production electron microscope stereology	990
porcine	discontinuous	testis	Purity of Leydig cells was > 85%.	effect of hydrocortisone (HS) and adrenocorticotropic hormone (ACTH) on testosterone production	991
rat	continuous	testis	Rat Leydig cells were purified from testis using elutriation followed by Percoll gradient centrifugation.	cell culture, the effect of human chorionic gonadotropin (hCG) on its gene regulation and protein secretion	992
rat	continuous	testis		cell culture, the effect of GH-releasing hormone (GHRH) on Leydig cell steroidogensis	993
rat	continuous	testis	Rat Leydig cells were purified from testis using elutriation followed by Percoll gradient centrifugation. Band 2 (of 3) contained > 95% Leydig cells (average density was 1.075 g/mL).	cell culture in presence of ^{125}I-labeled hCG, testosterone and cAMP production	994
rat	continuous	testis	Comparison of Leydig cells of different densities were made.	viability staining, cell culture	995
rat	continuous	testis		viability staining, *in vitro* testosterone production, SDS-PAGE electrophoresis	996
rat	continuous	testis	Isolation by Percoll gradient resulted in complete retention of morphological and biological integrity and a purity of 90 to 95%.	cell culture in presence of human chorionic gonadotropin (hCG), phase contrast microscopy, light microscopy and electron microscopy	31
rat	discontinuous (2-step)	testis		cell culture in the presence of interleukin-1 (IL-1)	997
rat	continuous (self-generating)	testis	Leydig cell precursors and pure (96%) Leydig cells were isolated on Percoll gradients.	cell culture in presence of human chorionic gonadotropin (hCG)	998
rat	discontinuous	testis	The purity of Leydig cells ranged from 90% to 95%.	cell culture in presence of human chorionic gonadotropin (hCG)	999
rat	discontinuous and continuous	testis	In the discontinuous gradient, the densest fraction contained a high proportion of Leydig cells whereas the lighter fraction contained mostly non-Leydig cells.	^{125}I-labeled iododeoxyuridine incorporation	1000

Spermatozoa

Species	Gradient type	Comments	Downstream application	Ref. #
bovine	discontinuous	Percoll was thought to improve semen and preserve acrosome integrity.	acrosome microscopy evaluation	1024
hamster	continuous	Caput epididymal spermatoazoa, with a specific gravity of 1.10–1.12 g/mL, were isolated without contamination by other cells.	lipid extraction and fractionation electron microscopy	1025
macaque	continuous	Percoll separation resulted in increased sperm-zona binding and did not affect the percentage of acrosome-reacted sperm bound to the zona or the percent motility and percentage of acrosome-reacted sperm in suspension.	zona binding experiments, acrosome reaction, motility assays	1026

Bone marrow cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
normal human	discontinuous (2-layer)	bone marrow	Megakaryocytes were at the interface between 1.020 g/mL and 1.050 g/mL.	magnetic beads for further purification, flow cytometry	1027
normal human	discontinuous	blood	B cells were recovered at least 95% pure. Gradients removed B-cell blasts very effectively.	flow cytometry	1028
HIV infected, normal and immune throm-bocyto-penic purpura human	discontinuous (2-layer)	bone marrow	Cells at the 1.020/1.050 interface were enriched 10-fold in megakaryocytes, while those at the 1.050/1.070 interface were immature cells.	megakaryocyte cultures prepared from immature cells for *in situ* hybridization	1029
normal human	discontinuous (2-layer)	bone marrow	Percoll density fractionation resulted in the depletion of greater than 95% of total marrow cells and an increase in megakaryocyte frequency from about 0.05% to 3% to 7%.	preparation of RNA and subsequent PCR, flow cytometry	1030
normal and arthritic human	discontinuous (3-layer)	bone marrow	Cells prepared were suitable for cell culture.	colony plaque assay, immunoflourscence, flow cytometry, protein colony blotting, RNA-colony blotting	1031
normal and leukemic human	discontinuous (4-layer)	peripheral blood	Low density cells post- and pre-transplant were prepared for analysis.	magnetic beads for further purification, PCR	1032
normal human	discontinuous (7-layer)	bone marrow	T cells obtained using Percoll were enriched about two-fold in the high-density fractions of marrow cells and depleted by about four- to five-fold in the lowest-density fraction as compared with Ficoll™ medium.	flow cytometry, mixed lymphocyte reaction assay, natural killer cell assay, cell culture	1033
normal human	discontinuous (1-layer)	bone marrow	Bone marrow cells were prepared using Percoll to remove RBC.	isolation of CD34+ cells using soybean agglutinin-coated flasks, progenitor cell assays, and flow cytometry	1034
marmoset	discontinuous (1-layer)	bone marrow	Bone marrow megakaryocytes from both interleukin-6 (IL-6) treated and untreated animals could be separated in Percoll.	flow cytometry	1035
primate	discontinuous (1-layer)	bone marrow	Bone marrow was isolated from both normal monkeys and interleukin-6 (IL-6) treated monkeys.	cell enumeration, FACS, digital imaging microscopy and electron microscopy	1036

Bone marrow cells (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
monkey	discontinuous (1-layer)	bone marrow peripheral and blood	Light density cells were prepared from aspirates over a 60% cushion.	cell culture and identification of various colony types	1037
mouse	discontinuous (1-layer)	bone marrow	Red blood cells were removed from bone-marrow preparations with a single 70% Percoll cushion.	culture of hematopoietic precursers, effects of interleukin-10 (IL-10) on proliferation, alkaline phosphatase activity, collagen synthesis assay, osteocalcin, preparation of RNA, and electron microscopy	1038
mouse	discontinuous (3-layer)	bone marrow	Bone marrow progenitor cells were suitable for culture.	effects of interleukin-3 (IL-3) and lipoplysaccharide (LPS) on cultured cells	1039
mouse	discontinuous (3-layerlayer)	bone marrow	Cells prepared were depleted of lymphoid and macrophage-lineage cells by addition of monoclonal antibody plus complement.	FACS analysis, hematopoietic progenitor cell culture, reconstitution of lethally irradiated mice	1040
mouse	discontinuous (3-layer)	bone marrow	Percoll was used to separate bone marrow fractions containing mostly blasts and lymphoid cells from those containing a high level of colony-forming units-spleen (CFU-S) counts.	FACS analysis, chemotaxis assay, assay of colony-forming units-spleen (CFU-S)	1041
mouse	discontinuous (3-layer)	protease-treated calvarial bone sections	Percoll gradients gave distinct subpopulations of cells based upon the results of various assays.	primary cell culture, flow cytometry, insulin-like growth factor I (IGF-I) assay, binding of epidermal growth factor, alkaline phosphatase determination	1042
mouse	discontinuous (4-layer)	bone marrow	Normal suppressor cell activity was maintained after separation.	suppressor cell activity assay	1043
mouse	discontinuous (4-layer)	bone marrow	Cells at a 1.06/1.07 g/mL density were used in subsequent studies.	reconstitution of lethally irradiated animals	1044
mouse	discontinuous (5-layer)	bone marrow, spleen		flow cytometry, reconstitution of lethally irradiated mice	1045
rat	discontinuous (3-layer)	bone marrow	About 75% of the input CFU-megakaryocytes (CFU-MK) were recovered in the fraction between 1.063 and 1.082 g/mL Percoll. CFU-MK were enriched only in this density fraction.	culture of hematopoietic progenitor cells	1046
rabbit	continuous	bone marrow		implantation into *in vivo* placed diffusion chamber, cytochemical staining, and electron microscopy	38
feline	discontinuous (1-layer)	bone marrow	Marrow mononuclear cells from both feline immunodeficiency virus-infected cats and normal cats were isolated.	culture of hematopoietic progenitor cells	1047

Macrophages

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	lung	Alveolar macrophages were purified from contaminating granulocytes using a discontinuous Percoll gradient.	superoxide (SO) release	1048
human	discontinuous (4-layer)	brochoalveolar lavage	Percoll gradients gave > 95% alveolar macrophage (AM) purity.	cell viability assay, light microscopy	1049
human	discontinuous (4-layer)	lung	Use of Percoll resulted in near total purification of alveolar macro-phages (AM) from other cells.	superoxide (SO) anion release	1050
human	discontinuous (4-layer)	decidual tissue	When cells were purified further with Percoll, the percentage of CD-14-positive cells increased by 52%.	secretion of platelet-activating factor (PAF) acetylhydrolase	1051
human	discontinuous	pulmonary	> 97% of the cells of fractions 1 to 4 were (4-layer) shown to be alveolar macro-phages (AM) in a previous study.	nonspecific esterase staining, flow cytometric DNA analysis	1052
human	discontinuous (4-layer)	lung	This method was used to study alveolar macrophage (AM) heterogeneity. The increased numbers of hypodense AM found in the asthmatic patients were unlikely to be due to the procedure.	cell viability, esterase and peroxidase activity assays, electron microscopy, generation of superoxide anion and thromboxane B2	1053
human	discontinuous (5-layer)	peripheral blood	Percoll-isolated monocyte/macro-phages were harvested from the top layer and routinely contained 75%/90% monocytes/macrophages as identified by Wright-Giemsa stain.	interactions between monocyte/macrophage and vascular smooth muscle cells	928
mouse	continuous and discontinuous	peritoneum	The total cell yield was 100.0% ±0.8%, and as measured by the trypan blue exlusion test, the cell viability was completely preserved.	light microscopy, trypan blue exclusion, esterase activity assay, peroxidase activity assay, cell immunophenotyping, bacterial phagocytic assays	1054
mouse	discontinuous (4-layer)	cultured cells	Percoll did not have a detectable effect on the cytolytic activity of cultured macrophages or on their viability.	phagocytic and cytolytic assays	30
mouse, rat	continuous and discontinuous	peritoneum	A continuous gradient followed by a discontinuous gradient was used to isolate all cell populations according to their actual density. This procedure yielded cells of high viability with preservation of critical cell function.	trypan blue exclusion	1055
rat	discontinuous (5-layer)	lung	The Percoll fractions were designated I to IV in order of increasing density with a percent distribution of cells of about 5%, 15%, 50% and 30%, respectively. Cell viability was > 95%.	fluorescence microscopy	1056

Macrophages (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
rat	discontinuous (5-layer)	lung	Cell viability was > 95% by trypan blue exclusion and > 95% were identified as alveolar macrophages (AM) in un-fractionated and fractionated cells by Giemsa and nonspecific esterase stains.	effects of pulmonary surfactant and protein A on phagocytosis, light microscopy	1057
rat	continuous	broncho- alveolar lavage	The various fractions comprised approximately 90% to 99% macrophages in virtually all instances.	esterase activity, surface expansion of Ia antigen by an immunoperoxidase technique	1058

Mast cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mouse	NA	peritoneum	Purity of the mast cells was nearly 100%, as checked by Memacolor fast staining.	qualitative and quantitative PCR analysis	1059
mouse	continuous	peritoneum	Starting from a peritoneal cell population containing 4% mast cells, a mast cell purification of up to 95% was obtained.	electron microscopy and ultrastructural cytochemistry	8
rat	discontinuous	peritoneum	Mast cell purity with Percoll was > 95%.	direct interaction between mast and non-mast cells, histamine release assay	1060
rat	continuous	peritoneum	Mast cells purified on Percoll gradients were more than 90% pure by toluidine blue staining, and the viability was > 98% by the trypan blue exclusion test.	flourometric assay to measure histamine release	1061
rat	continuous	peritoneum	Mast cells can be isolated with high yields and purity by centrifugation on gradients of Percoll.	light and electron microscopy, cytofluorometry	9
rat	continuous (sequential)	peritoneum	The purity of mast cells purified over sequential Percoll gradients was evaluated by measurement of the contribution of eosinophil peroxidase to mast cell peroxidase activity.	histamine release and peroxidase activity	1062

Thymocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mouse	discontinuous (5-layer)	thymus	Percoll was used for separation of immature thymocytes.	*in vitro* stimulation by mitogens, isolation of nuclei, isolation and gel electrophoresis DNA, enzyme assays	1063
rat	discontinuous	thymus	Percoll was used for separation of normal and apoptotic thymocytes.	flow cytometry	1064
rat	discontinuous (4-layer)	thymus	Percoll was used for separation of cells possessing the characteristically condensed nuclear chromatin associated with apoptosis from apparently normal thymocytes.	electron microscopy, Coulter counter analysis, flow cytometry, DNA analysis	1065
rat	discontinuous (4-layer)	thymus	Percoll was used for isolation of a transitional population of pre-apoptotic thymocytes.	DNA analysis, isolation of nuclei and DNA autodigestion, light and electron microscopy	1066
rat, mouse	discontinuous (3-layer)	thymus	Percoll was used to separate large and small thymocytes. An extremely high level of viability was maintained.	phase contrast microscopy and autoradiography	62

Miscellaneous cells

Cell type	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
pancreatic islets	human, mouse	continuous	pancreas	The use of Percoll eliminated the problems of high viscosity, undesired osmotic properties and, in some cases, also toxic effects.	density determination and insulin secretion	5
endothelial	human	continuous linear gradient	whole blood	Final recovery of endothelial cells was 91.6%.	immunofluorescence	1067
trophoblasts	rat	continuous	placenta	Percoll gradient centrifugation yielded efficient separation of rat placental lactogen-II (rPL-II) producing cells from digested tissue from labyrinth and junctional zones of the chorioallantoic placenta.	development of *in vitro* rat placental trophoblast cell culture system	1068
various	NA	NA	NA	This paper compared different approaches to cell separation. According to the authors, Percoll is generally the most useful media for isopycnic centrifugation of most kinds of cells.	none	1069

Miscellaneous cells (continued)

Cell type	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
viable vs. nonviable	human, rat	discontinuous (2-layer)	various tumor tissue	Interface showed a viability of > 90%, but the yield of viable cells decreased dramatically if the tissue resection was not immediately processed.	trypan blue viability assay, 2-D PAGE	1070
apoptotic	human	discontinuous (7-layer)	promyelocytic leukemic cell line	The step gradient used generated three main cell bands and a cell pellet, the pellet was very enriched for apoptotic cells (85% to 90%).	DNA isolation	1071
lymphoblast	human	continuous	whole blood	Lymphoblasts were enucleated using a Percoll gradient containing cytochalasin B.	electrofusion	1072
brain capillary endothelial	rat	continuous pre-made	brain	Subsequent Percoll gradient centrifugation resulted in a homogenous population of capillary endothelial cells capable of attachment to collagen and incorporation of tritiated thymidine.	cell culture, light microscopy electron microscopy	170
neurons	rabbit	discontinuous and rate zonal	dorsal-root ganglia	Neurons were isolated with a viability of 80% and a purity of > 90%.	cell culture, light and electron microscopy	480
non-myogenic separated from myogenic	chicken	discontinuous	breast muscles	Separation of cells from embryonic muscle allowed direct analysis of cell-specific proteins without the need for cell culturing.	cell culture, microscopy, DNA/protein analysis	680
megakaryocytes	human	discontinuous	bone marrow	Isolation of megakaryocytes was reproducibly better in Percoll than in BSA.	Ficoll 400 centrifugation to further purify, complement receptor assay	155
chondrocytes	rat	discontinuous	bone marrow	Cell viability was > 95% while yield varied depending on aggregation of cells.	cell culture, quantitation of proteoglycans and collagen	629
spermiophages	turkey	discontinuous	sperm	Spermiophages fixed immediately after Percoll isolation resembled those in freshly ejaculated semen except for an apparent increase in the number of mitochondria.	light and electron microscopy, cell culture	1073
NA	human	continuous	parathyroid gland	Densities of parathyroid glands were measured using various density gradient media. For densities > 1.0 g/mL, Percoll proved superior to any of the other gradient liquids investigated.	glandular density determination	2

Applications — microorganisms

Microorganisms

Species	Type	Gradient type	Host tissue	Comments	Downstream application	Ref. #
Bacteroides sp.	bacteria	discontinuous (4-layer)	NA	Percoll was used to assess the degree of capsulation of the twelve *Bacteroides* strains grown in three different media.	light microscopy	1074
Ehrlichia ristcii	bacteria	continuous	cultured cells	Percoll was used to purify *Ehrlichia risticii* from an infected murine macrophage cell line (P388D).	CO_2 production assay, Coomassie brilliant blue dye binding assay	1075
Ehrlichia risticii	bacteria	continuous	cultured cells	*Ehlichia risticii* was purified from an infected murine macro-phage cell line (P388D).	CO_2 production assay, Coomassie brilliant blue dye binding assay	1076
Porphyromonas gingivalis	bacteria	continuous	NA	Percoll was used to separate unbound cells from saliva-coated bead (SHAP)-bound cells.	binding and binding inhibition assays	1077
Treponema pallidum	bacteria	continuous	NA	Percoll-purified treponemes from 5-day infections were immobilized significantly more slowly than the purified trepo-nemes from 7- and 8-day infections.	influence of different sera on *in vitro* immobilization of Percoll-purified *Treponema pallidum*	1078
Theileria sp.	bacteria	discontinuous (2-layer)	bovine erythrocytes	A purification method for viels from *Theileria*-infected bovine erythrocytes was developed.	light and electron microscopy and 1- and 2-D poly-acrylamide gel electrophoresis	1079
Babesia bigemina	protozoa	continuous and discontinuous (4-layer)	bovine erythrocytes	*Babesis bigemina*-infected erythrocytes were successfully concentrated at least 20 times by Percoll and Percoll-Renografin density gradients.	enzymatic studies and starch gel electrophoresis	1080
Babesia equi	protozoa	continuous	horse erythrocytes	The piroplasms of *Babesia equi* were purified by lysis of infected horse erythrocytes and Percoll density-gradient centrifugation.	protein characterization of *B. equi* piroplasms	1081
Plasmodium berghei and P. chabundi	protozoa	continuous	mouse blood	Percoll was used for the separation of host erythrocyte membrane from malarial parasites. The recovery of the erythrocyte membranes was ~65% to 70%, whereas parasite re-covery was 80% to 90%, and the relative purity was ~85% to 90%.	electron microscopy, electro-phoresis, immuno-blotting, marker enzyme analysis and pulse chase analysis	1082
Babesia bovis	protozoa	continuous	bovine erythrocytes	A 65% Percoll concentration was found to be optimal for *Babesia bovis* merozoite (i.e., mature exoerythrocytic stage) separation. A 100% Percoll stock solution was optimal for enrichment of infected erythrocytes.	parasite viability assay	1083

Microorganisms (continued)

Species	Type	Gradient type	Host tissue	Comments	Downstream application	Ref. #
Entamoeba histolytica	protozoa	discontinuous (2-layer)	faecal cyst	Percoll purification provided a good yield even from a moderate faecal cyst load in a single stool sample.	*E. histolytica* for use as antigen	1084
Vairimorpha necatrix	protozoa	continuous	caterpillar	Percoll was used to purify spores. 40% of the original spores were recovered with nearly all refractile (90% or more). Contaminating bacteria were not seen.	infection of cultured cells	1085
rice transitory yellowing virus (RTYV)	virus	continuous	rice plant leaf	Typical purification runs gave about 140 to 850 mg of purified virus per 100 g of infected material.	Lowry protein assay, electron microscopy, SDS-PAGE, ELISAs, Western blots	1086
Rubivirus (rubella virus)	virus	continuous	cultured cells	Comparison of Percoll and sucrose gradients for purifying *Rubella* gave a yield of 72% with Percoll compared to 8.6% with a sucrose gradient.	hemagglutinating titer assays	1087
Herpes simplex virus	virus	continuous	NA	Percoll was used to purify herpes simplex virus.	none	56
dino-flagellates, diatoms, blue-green bacteria	marine micro-algae	continuous	NA	Most of the marine species recovered were in a condition that would permit direct physiological measurements of photosynthesis, respiration, ion adsorption and specific growth rates.	light microscopy, motility assay, photosynthesis assay	60
mycoplasma-like organism (MLO)	NA	discontinuous	lettuce (*Lactuca sativa*)	Electron microscopy showed a high concentration of MLOs with well-preserved cellular structures.	electron microscopy, ELISA	1088

Applications — subcellular particles

Plasma membranes

Species	Gradient type	Tissue/Cell type	Comments	Downstream application	Ref. #
human	continuous (self-generating)	platelets	A method for rapid isolation of platelet plasma membrane was described, based on the use of [^{3}H]-concanavalin A as a membrane marker and self-generating gradients of Percoll.	radioactive tracer studies, enzyme and protein assays	54
rat, human	continuous	liver biopsy	Plasma membrane enzymatic marker and membrane transport assays indicated that isolated membranes retained their functional integrity.	membrane enzyme assays and measurement of amino acid transport by membrane vesicles	1089
rat	continuous	uterus	The plasma membrane markers, 5′-nucleotidase and cholesterol, were enriched in the fractions near the top of the gradient, while the sarcoplasmic reticulum marker enzyme, rotenone-insensitive NADH-cytochrome-c reductase, was in the lower part.	Ca++ uptake and release assays enzyme assays, cholesterol and progesterone assays, and Western blot	1090
rat	continuous (3-layer)	brain	Synaptic plasma membranes were prepared by Ficoll and Percoll density gradients.	phospolipase C assay, marker enzyme assays	1091
rat	discontinuous (2-layer)	cultured cells	Two subcellular fractions, one enriched in plasma membranes and the other enriched in endoplasmic reticulum membranes, were obtained by Percoll gradient fractionation.	electron microscopy, determination of enzymatic markers, enzyme activity, calcium uptake and release	1092
rat	continuous	liver	The plasma membrane marker, 5′-nucleotidase, was enriched, whereas the cytosolic (endoplasmic reticulum) enzyme, glucose-6-phosphatase, was impoverished, indicating vesicle purity.	vesicle amino acid transport assay	1093
rat	continuous	liver	Percoll gradients were used to isolate hepatocytes, plasma membranes and mitochondrial membranes.	phase-contrast microscopy, cell binding experiments	33
rat	continuous	liver	Use of Percoll for the low speed nuclear pellet resulted in plasma membrane markers and Ins (1,4,5)P3 binding activity being purified together.	marker enzyme determinations, Ins(1,4,5)P3 binding, Bradford protein assay, SDS-PAGE	1094
rat	continuous	liver	Percoll purified hepatic plasma membranes were used to examine the transport of amino acids.	arginine transport activity, enzyme marker assays	1095

Plasma membranes (continued)

Species	Gradient type	Tissue/Cell type	Comments	Downstream application	Ref. #
bovine	continuous	cultured aortic endothelial cells	Plasma membranes were labeled with trace amounts of [^{3}H]-cholesterol and cell homogenates were fractionated on sucrose and Percoll gradients.	enzyme assays and SDS-PAGE/ligand blots	1096
bovine	discontinuous (3-layer)	adrenal gland	The procedure provided a fraction rich in plasma membranes.	solubilization of plasma membranes, affinity chromatography, radiolabeling of plasma membrane, enzyme assays	78
sheep	continuous (self-generating)	perirenal fat adipocytes	The fatty acid content of plasma membranes was analyzed.	fatty acid analysis using gas-chromatography	1097
Chinese hamster	continuous (self-generating)	cultured chinese hamster ovary (CHO) cells	A procedure yielded plasma membrane fractions that were enriched 3-fold and practically free of lysosomes; pure endoplasmic reticulum (ER) and mitochondrial fractions were obtained as well.	lipid analysis, enzyme assays	1098
skate *(Raja erinacea)*	continuous (self-generating)	liver	Marker enzyme studies indicated that plasma membranes isolated with Percoll gradients were highly enriched in the basolateral domain of the liver plasma membrane and largely free of contamination by intracellular organelles or canalicular membranes.	enzyme assays, fluorescence anisotropy measurements, alanine transport, protein and lipid determination	1099
Fungi *(Penicillium chrysogenum)*	continuous	NA	The majority of contaminating membranes were removed by Percoll step gradients.	enzyme assays, electron microscopy, membrane fusion, transport studies, Lowry protein assay	1100
Fungi *(Penicillium cyclopium)*	continuous	NA	Right-side-out plasma membrane vesicles were prepared using two-phase partitioning and Percoll gradients.	ATPase activities, electron microscopy	1101

Lysosomes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	cultured fibroblasts	Only lysosomes, sedimented in the bottom third of 30% to 40% Percoll density gradients.	adenosine deaminase and N-acetyl-b-hexosaminidase assays	1102
human	continuous	cultured fibroblasts	A crude mitochondrial lysosomal pre-paration of fibroblasts was separated into high-density fractions (lysosomal markers) and low-density fractions (mitochondrial markers).	enzyme assays, SDS-PAGE electrophoresis, immunoblotting	1103
mouse	continuous	liver	After homogenization, lysosomes equilibrated in the dense regions of Percoll gradients.	electron microscopy, Bradford protein assay, enzymatic assays	1104
rat	continuous	cultured hepatocytes	Lysosomal fractions were used to assay for endocytic transport of lysosomal membrane glycoprotein from cell surface to lysosomes.	purification of lysosomal membrane glycoprotein, Lowry protein assay, protein-horseradish peroxidase assay	1105
rat	continuous (self-generating)	liver	Analysis of relevant marker enzymes showed considerably purified lysosomal particles in the density range of 1.04 to 1.11 g/mL.	Lowry protein assay, enzyme assays, free isoelectron focusing	24
rat, buffalo	continuous (differential and isopycnic)	kidney	The method gave a 25 to 40-fold enrichment in lysosomal marker enzymes with < 0.5% contamination from mitochondrial and peroxisomal markers.	preparation of membrane vesicles, electron microscopy, protein assay	1106
porcine	continuous	cultured kidney epithelial cells	The method allowed for the relatively easy preparation of enriched fractions of endosomes and lysosomes.	distribution and structure of vacuolar H^+ ATPase, radiolabeling detection, hexosaminidase activity and alkaline phosphatase activity	1107

Mitochondria

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
plant	discontinuous	etiolated tissue and green leaf tissue	For etiolated tissue mitochondria, about 90% of catalase contamination was removed. For green leaf mitochondria, about 95% of chlorophyll, 80% of catalase and 65% of glycollate oxidase were removed.	cytochrome c oxidase (CCO) activity, membrane activity, respiratory control and substrate oxidation measurements	12
plant	discontinuous (3-layer)	etiolated tissue and green leaf tissue	Separation of mitochondria from chloroplast material was possible under isoosmotic conditions, and in a relatively short time.	chlorophyll, cytochrome c oxidase and glycollate oxidase activities	43
rabbit, porcine	discontinuous	heart	Percoll was especially suitable for *in vitro* studies on mitochondria from both normal and diseased hearts.	electron microscopy, enzyme activities	1108
rat	discontinuous	liver	Isolated rat liver mitochondria were split into three density fractions when applied to a Percoll gradient.	staining of mitochondrial populations, flow cytometry	1109
Plasmo-dium berghei (protozoa)	continuous	NA	The purified mitochondria were obtained at the interface with a density of 1.05 g/mL.	mitochondrial marker enzyme assays, phase-contrast and electron microscopy	1110
turkey	discontinuous (3-layer)	sperm	Mechanical disruption, sonication and centrifugation over Percoll was an effective procedure to isolate the mitochondria.	fluorescence and electron microscopy, cytochrome oxidase assay, oxygen consumption, mitochondrial DNA isolation	1111

Granules

Cell type	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (3-layer)	whole blood	neutrophils	Specific and gelatinase granules were separated on a three-layer Percoll gradient.	myeloperoxidase, alkaline phosphatase, lactoferrin, gelatinase, B12 binding protein, β2 micro- globulin, cytochrome b558, and CD116 assays	1112
human	discontinuous (2-layer)	whole blood	neutrophils	Subcellular fractionation resulted in a band containing gelatinase and specific granules and a band containing plasma membrane and secretory vessels.	receptor localization, enzyme marker assays	1113
human	discontinuous (2-layer)	whole blood	neutrophils	Percoll gradient centrifugation resulted in a bottom band containing azurophil granules, a top band of plasma membrane and secretory vesicles, and a clear super-natant containing cytosol.	marker enzyme assays, ELISA	1114
human	discontinuous (2-layer)	whole blood	neutrophils	Percoll was used for subcellular fractionation of plasma membranes, specific granules and azurophilic granules.	subcellular localization of myeloperoxidase alkaline phosphatase, and vitamin B12 binding protein	1115
human	continuous	whole blood	primary cultured lymphocytes	Percoll gradients were used for the isolation of large granular lymphocyte (LGL) cytoplasmic granules.	macrophage tumorcidal assay	1116
mouse	continuous	mastocytoma	mast cell	Density gradient centrifugation was carried out in Percoll/0.25 M sucrose.	uptake and degradation of mast cell granules by mouse peritoneal macrophages	17
rat	continuous	parotid gland	NA	A secretory granular fraction (SG) and a plasma membrane-rich fraction (PM) were isolated using differential and Percoll gradient centrifugation.	enzyme assays, interactions of SG with PM	1117
bovine	discontinuous (3-layer)	adrenal gland	NA	Using Percoll to isolate chromaffin granules did not increase the yield, but it did eliminate the need for exposure of the granules to extreme hypertonic conditions during isolation.	electron microscopy, glutaraldelyde fixation for preparation of affinity column	78
Paracentrotus lividus (sea urchin)	discontinuous (2-layer)	NA	NA	Lytic molecules were contained within small (0.1 to 0.25 mm) granules (cytolytic granules) which could be isolated by Percoll gradients.	hemolytic and enzymatic activities	1118

Plant organelles

Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mitochondria	castor bean	continuous	seed (endosperm)	Highly purified mitochondria were obtained with the Percoll gradient.	mitochondrial cytidyl-transferase assay	1119
mitochondria	sunflower	continuous	seed	No organellar contamination was seen in the pellet sections.	Lowry protein assay, characterization of NADP-dependent isocitrate dehydro-genase (NADP-IDH), SDS-PAGE and native gel electrophoresis, gel filtration, electron microscopy	1120
mitochondria	maize, faba bean, wheat, tobacco, sugar beet	discontinuous	leaf	The purified intact mitochondria exhibited high respiratory controls and P/O ratios and were cleared of most of the chlorophyll.	*in vitro* radioactive labeling of the products of mitochondrial protein synthesis and their analysis by SDS-PAGE	1121
chloroplast	tobacco *(Nicotiana tabacum)*	discontinuous	leaf	The yield from the Percoll gradient was 4.63×10^7 chloroplasts/g of chlorophyll/chloroplast.	Extraction of chloroplast proteins, Bradford protein assay, SDS-PAGE, protein blotting and immunological reactions	1122
chloroplast	Pea (*Pisum sativum*) and spinach (*Spinacea oleracea*)	continuous	leaf	The purified chloroplasts were capable of light-dependent protein synthesis at rates comparable to those previously reported.	*in vitro* reconstitution of protein transport and fractionation of chloroplast stromal protein	76
chloroplast	spinach (*Spinacea oleracea*)	continuous linear gradient	leaf	A clear separation of intact chloroplasts sustaining high photosynthetic activities occured.	enzyme assays, photosynthetic CO_2 fixation, and O_2 evolution	88
cytoplasts	various	discontinuous	leaf	Cytoplasts were obtained by centrifugation of leaf protoplasts on Percoll gradients.	cytoplast staining, laser microscopy, cytoplast: protoplast fusion	1123
protoplasts	barley	discontinuous (4-layer)	seed (aleurone layer)	After the Percoll gradient, the protoplasts were obtained in relatively high yield and showed good viability.	transient expression of CAT activity by transfected barley protoplasts	1124
nuclei	carrot	discontinuous (3-layer)	suspension cells	This method yielded an average of 2×10^5 nuclei from 2 g of suspension cultured cells (approximately 2×10^6 cells). Greater than 80% of the nuclei appeared fully intact following the Percoll gradient.	cytochrome c oxidase and reductase assays, and *in vitro* RNA synthesis	1125
plastids	barley, pea, maize	continuous	leaf and seed endosperm	Plastids obtained using Percoll exhibited high degrees of intactness (89.1% and greater) and purity.	starch synthesis, enzyme assays	1126

Miscellaneous organelles

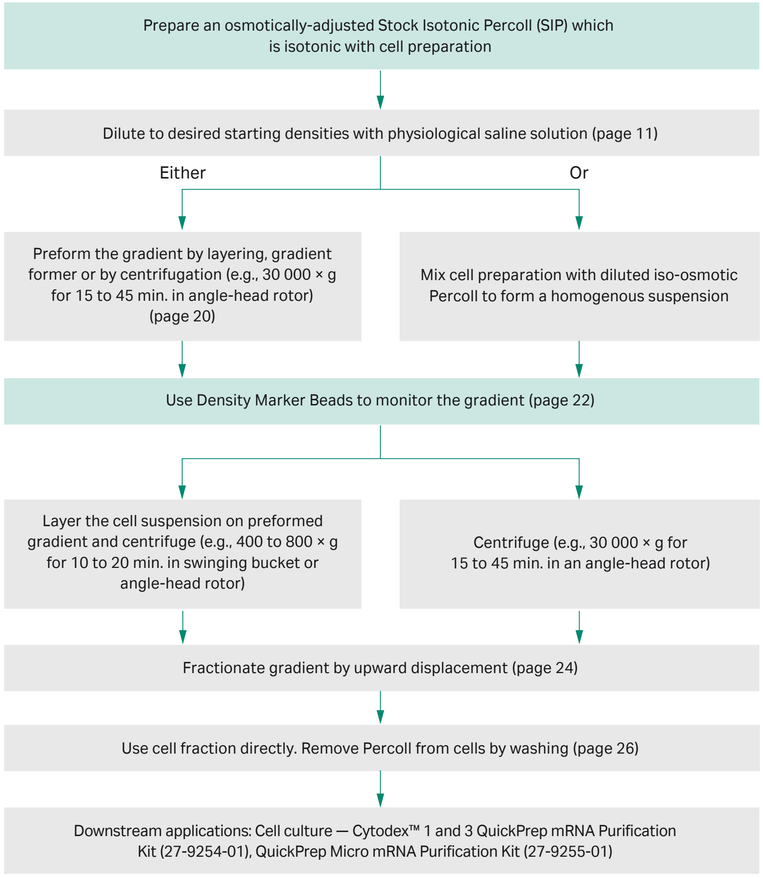
Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
nuclei	chicken	continuous (self-generated)	skeletal muscle	Percoll density gradient centrifugation provided a convenient method for the isolation of transcriptionally active nuclei applicable to a variety of tissues.	*in vitro* transcription	832
nuclei	*Neurospora crassa* (fungi)	continuous	whole organism	Percoll was a very effective alternative to LUDOX™ for the purification of *Neurospora* nuclei from crude nuclear preparations.	electron microscopy, DNA, RNA and protein purification	1127
nuclei and sub-cellular fractionation	NA	continuous (self-generated)	cultured NIH and KNIH cells	Percoll centrifugation allowed efficient fractionation and preservation of enzymatic activity.	β-galactosidase and galactosyltransferase activity	597
endosomes	human	continuous	cultured hepatoma cells	Percoll gradients were used to separate endosomes from lysosomes. The conditions of centrifugation were chosen specifically to permit resolution of early, intermediate and late endosomes.	β-hexosaminidase activity, Bradford protein assay	1130
endosomes	human	continuous	cultured B cells	Percoll was used to isolate intracellular major histocompatability complex (MHC) molecules in a preparative scale from endosomal compartments.	sequence analysis of pooled and single peptides, fluorescence labeling and binding assay	1131
plasma membrane, endoplasmic reticulum, lysosomes and mitochondria	human	continuous	liver biopsy	Percoll permited rapid analytical subcellular fractionation. Resolution of organelles was good, and recoveries were high (86% to 105%).	marker enzyme assays	13
melanosomes, lysosomes, peroxisomes	human	continuous	cultured melanocytes	Subcellular fractionation was used to determine the relationship between melanosomes, lysosomes and peroxisomes.	enzyme activity assays, immunoflourescence and immuno-electron microscopy	1132
azygospores	*Condiobolas obscuros* (fungi)	discontinuous	whole organism isolated from soil	Recovery was 64% on average for a variety of soil types.	microscopy	246
chromosomal and mitotic clusters	human	continuous and discontinuous (self-generated)	cultured HeLa 53 and CHO cells	Chromosomes were isolated free of cytoplasmic contamination.	microscopy, Western blotting	677
peroxisomes	rat	continuous and discontinuous (self-generated)	liver		enzyme assay, fatty acid oxidation studies	53

Miscellaneous organelles (continued)

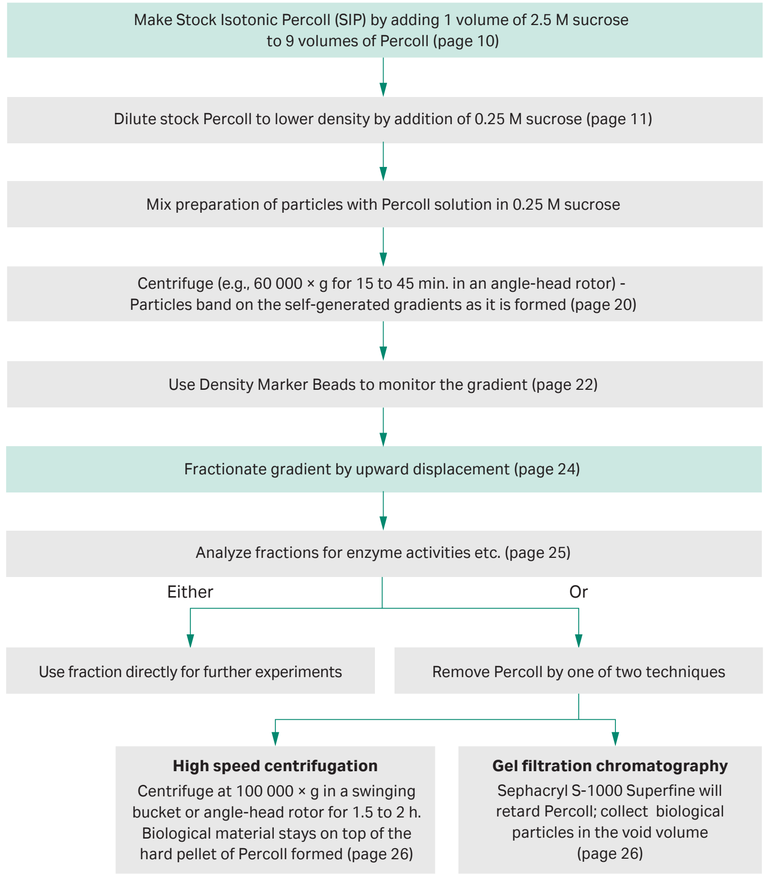
Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
various	human	continuous (self-generated)	blood	Percoll-sucrose gradients were used to purify subcellular fractions to assay for catalase.	indirect immunocytofluorescence microscopy, ultrastructural immunogold, enzyme activity assays	1133
cytosol, lysosomes, Golgi elements	NA	continuous (self-generated)	cultured monoblastic cell line	Percoll gradients were used to separate subcellular organelles into various fractions.	marker enzyme assays	1134
microbodies	*Cladosporium resinae* (fungi)	discontinuous	whole organism	Best results were obtained with a discontinuous Percoll gradient which yielded a fraction enriched in microbodies and one enriched in mitochondria.	catalase and cytochrome oxidase assays	1135
subcellular fractionation	human	continuous (self-generated)	cultured HL-60 cells	Percoll centrifugation allowed efficient fractionation and preservation of enzymatic activity.	peroxidase, β-glucuronidase and acid phosphatase assays	727
lipid vesicles	*Torpedo californica*	continuous (self-generated)	electroplax tissue	Intact vesicles were isolated.	phosphate determination	392

Appendix
Summary methodology charts

Scheme 1. Separation of cells on gradients of Percoll.



Scheme 2. Separation of subcellular particles and some viruses on gradients of Percoll.



References

Note: In portions of the list of references below, the numbering is not sequential. This is due to the way in which the list was constructed. All references with numbers lower than 891 have been extracted from the original Percoll Reference List (1992), and the numbering used in that List was maintained in this Manual. All references higher than 891 are new and are sequential.

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Products for cell separation and culture

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Percoll	1 l	17-0891-01
	250 mL	17-0891-02
Percoll PLUS	1 l	17-5445-01
	250 mL	17-5445-02
Ficoll-Paque PLUS	6 × 100 mL	17-1440-02
	6 × 500 mL	17-1440-03
Ficoll-Paque PREMIUM	6 × 100 mL	17-5442-02
	6 × 500 mL	17-5442-03
Ficoll PM 70	100 g	17-0310-10
	500 g	17-0310-50
	5 kg	17-0310-05
Ficoll PM 400	100 g	17-0300-10
	500 g	17-0300-50
	5 kg	17-0300-05
	40 kg	17-0300-08
Cytodex 1	25 g	17-0448-01
Cytodex 3	10 g	17-0485-01

Products for purification of RNA

Production	Quantity	Code number
QuickPrep *Micro* mRNA Purification Kit (24 purifications)	1 kit†	27-9255-01
QuickPrep mRNA Purification Kit (4 purifications)	1 kit†	27-9254-01
mRNA Purification Kit		
(2 purifications)	1 kit†	27-9258-01
(4 purifications)	1 kit†	27-9258-02
CsTFA (Solution)	100 mL	17-0847-02
Oligo(dT)-Cellulose Type 7	1 g	27-5543-02

Kits for cDNA synthesis

Product	Quantity	Code number
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First-Strand cDNA Synthesis Kit (55 reactions)	1 kit†	27-9261-01
Ready-To-Go™ T-Primed First-Strand Kit (50 reactions)	1 kit†	27-9263-01

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