

Ficoll PM70

Ficoll PM400

CELL PREPARATION

Ficoll™ PM70 and Ficoll PM400 are high molecular weight sucrose-polymers formed by copolymerization of sucrose with epichlorohydrin. The molecules are highly branched and the high content of hydroxyl groups leads to very good solubility in aqueous media. Ficoll PM70 and PM400 are supplied as spray-dried powders. Ficoll products behave as ideal neutral spheres and have been proposed as the molecules of choice for studying pore size distribution and the permeability of membranes. Ficoll PM70 and Ficoll PM400 have analogous structures, but differ in molecular weight, and are therefore appropriate for different research applications.

Stability

The stability of Ficoll products is chiefly determined by the glycosidic bonds in the sucrose residues. Ficoll products do not contain any ionized groups, so the structures do not react under physiological conditions. They are stable in alkaline and neutral solutions, but are rapidly hydrolyzed in solution at pH 3, especially at elevated temperature. Ficoll products can be sterilized by autoclaving at 110°C for 30 min in neutral solutions. Strong oxidizing and reducing agents should be avoided. Shipping and storage are at ambient temperatures.

Chemical and physical properties

Ficoll products are provided as dry powder and are extremely hydrophilic. Solutions are best prepared by slowly stirring Ficoll powder into aqueous buffer. Gentle heating may be required for complete solubilization.

Ficoll PM70 can be used at concentrations of up to 50% (w/v). Figure 2 shows the variation of viscosity of Ficoll PM70 with concentration at 20°C. Note that the viscosity of a Ficoll PM70 solution is rather less than that of a solution of dextran (M_r 70 000) having the same osmotic pressure. Figure 3 shows the variation of osmolality with the concentration of Ficoll PM70.



Fig 1. Ficoll PM70 and Ficoll PM400.

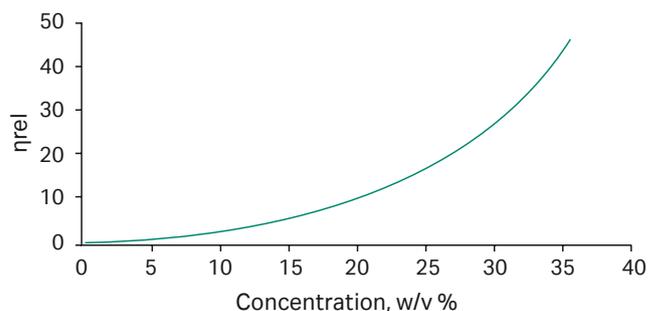


Fig 2. Variation of viscosity with concentration for solutions of Ficoll PM70 at 20°C.

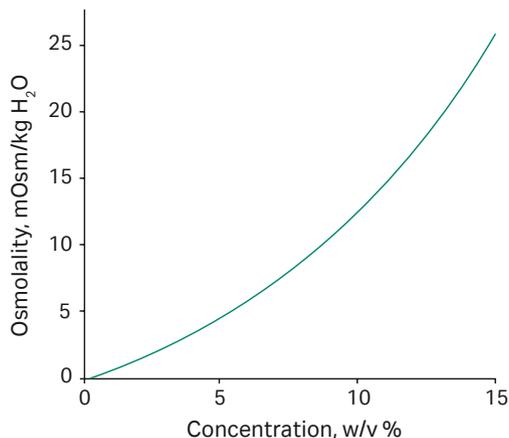


Fig 3. Variation of osmolality with concentration for solutions of Ficoll PM70 at 25°C.

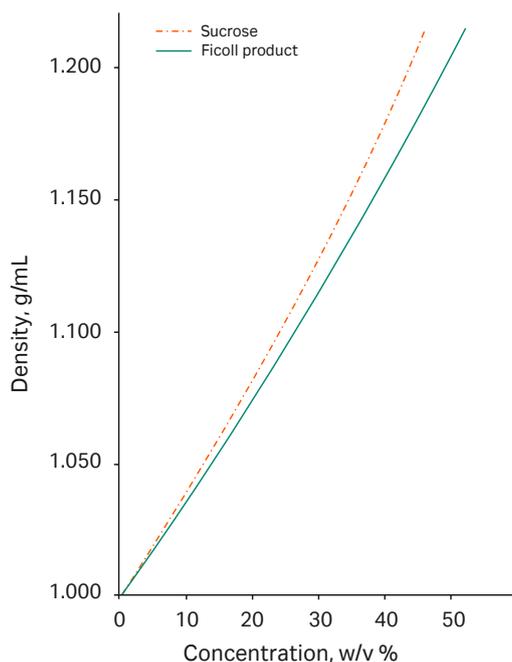


Fig 4. Densities of Ficoll PM400 and sucrose solutions as a function of concentration.

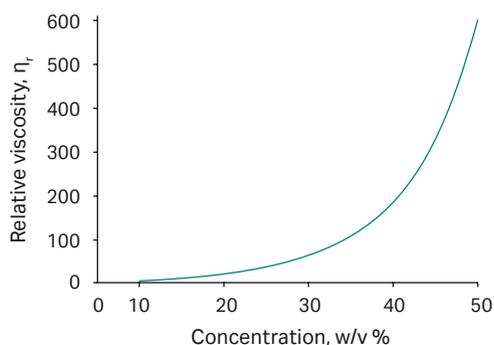


Fig 5. Relative viscosity (η_r) of Ficoll PM400 solutions at different concentrations.

With Ficoll PM400 one can also obtain concentrations of up to 50% (w/v) to cover a density range with a maximum of 1.2 g/mL, without exceeding normal physiological osmolality. Figure 4 shows the comparable densities of solutions of Ficoll PM400 and sucrose as a function of concentration. Figure 5 shows the relative viscosity (η_r) at 20°C of Ficoll PM400 solutions at different concentrations.

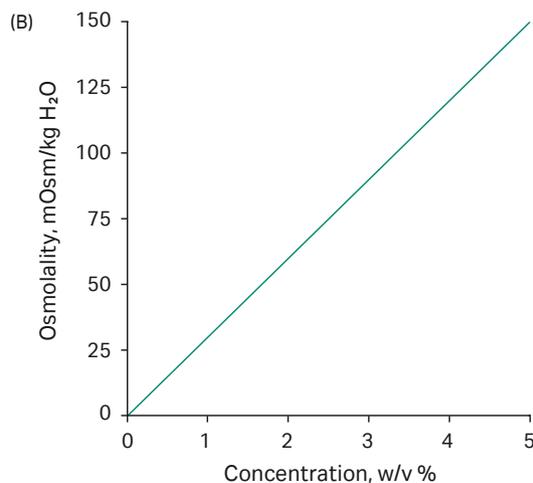
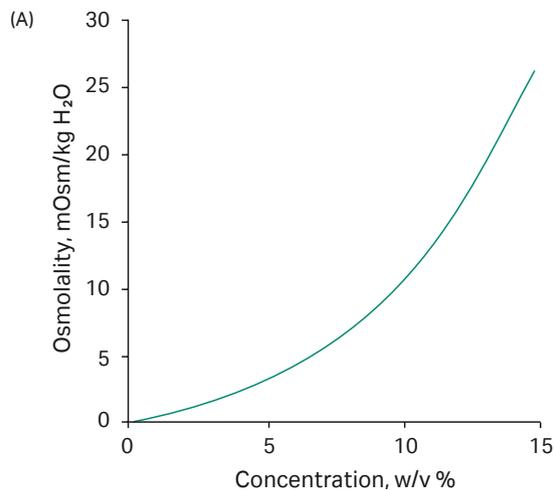


Fig 6. Variation of osmolality with concentration for solutions of (A) Ficoll PM400 and (B) sucrose at 25°C.

Solutions of Ficoll PM400 have much lower osmotic pressures than sucrose solutions at equivalent concentrations. Figures 6 (A) and 6 (B) show the variation of osmolality with concentration for Ficoll PM400 and sucrose respectively (note the considerable difference between the scales used in the two figures). The low osmolality of Ficoll products allows the formation of isotonic density gradients and preserves physiological and morphological integrity during centrifugation.

You can estimate the concentration of both Ficoll PM70 and Ficoll PM400 in aqueous solutions using the anthrone reaction (1).

Ficoll PM70

- Displays minimal toxicity
- Has favorable viscosity and osmotic properties (2)

Description

Cytiva produces a fraction of Ficoll product with an average molecular weight of 70 000. This material, Ficoll PM70, is specifically intended for use in perfusion media for laboratory studies on dextran-sensitive animals.

Technical data

Molecular weight range	~ 6×10^4 to ~ 8×10^4
Intrinsic viscosity	~ 10 mL/g
Specific rotation $[\alpha]^{20}$	+ 56.5°
Stokes radius	~ 5.1 nm
Cl- content	< 1%

Applications

Laboratory studies of the circulation system in animals frequently involve the use of artificial perfusion solutions of well-defined characteristics. To provide a suitable colloid osmotic pressure during the experiment, a high molecular weight polymer, frequently dextran, is added to the solution. The use of dextran for perfusion studies on rats is, however, not practically possible since infusion of dextran solutions produces anaphylactoid-like reactions in these animals (3). Ficoll PM70 has been specifically produced to make possible perfusion experiments using dextran-sensitive animals.

Studies of the circulation in spontaneously hypertensive and normotensive rats using this technique have been reported by Folkow *et al* (4–8). A 4% (w/v) solution of Ficoll PM70 was used to provide a normal colloid osmotic pressure (7) and both whole animal (4) and isolated hindquarter preparations (5) were used. The results of these experiments have provided evidence for morphological adaptations of the resistance vessels of the systemic circuit in hypertensive rats (4,5) which may be sufficient to account for their increased flow resistance during rest.

Ficoll PM70 has also proved valuable for cell isolation by unit gravity sedimentation (9).

Ficoll PM400

- Gives density range with a maximum of 1.2 g/mL, allowing the isolation of many types of cells and organelles (see Table 1)
- Has lower osmotic pressures than sucrose solutions of equal density, resulting in better preservation of the functional and morphological integrity of cells and organelles

Description

In a variety of research situations inert, non ionized polymers of high molecular weight are needed. Ficoll PM400 is a synthetic neutral, highly-branched hydrophilic polymer of sucrose with an average molecular weight of 400 000. It has long been used in the formation of density gradients for the separation and isolation of eukaryotic cells, organelles, and bacterial cells, as a stabilizing agent and as a separation medium for the isolation of lymphocytes. It also has applications in defined culture media, nucleic acid hybridization, electrophoresis, and immunological studies.

The favorable osmotic, density, and viscosity characteristics of Ficoll PM400 make it an excellent choice for many applications.

Because of its high molecular weight (~ 400 000) and low content of dialyzable material, Ficoll PM400 does not normally penetrate biological membranes.

Technical data

Molecular weight range	~ 3×10^5 to ~ 5×10^5
Intrinsic viscosity	~ 17 mL/g
Specific rotation $[\alpha]^{20}$	+ 56.5°
Stokes radius	~ 10 nm
Cl- content	< 1%

Applications

Centrifugation

In centrifugation methods, the density and viscosity of the medium are adjusted to allow particle sedimentation with a convenient speed. With sucrose, the high osmotic pressure, which results from the concentrations used, often damages the cells. If instead you add a high molecular weight polymer such as Ficoll product, you obtain the required density without significantly increasing the osmotic pressure. This preserves cells intact and retains their viability. Ficoll product is therefore preferred to sucrose for forming density gradients, and is primarily used in this way for the routine separation of cells (10, 11, 12)

Ficoll PM400 can be used for gradient centrifugation in all types of centrifuge rotors and for separation at unit gravity. For centrifugation, both discontinuous and continuous gradients are possible. Discontinuous gradients offer two main advantages: First, the abrupt changes in Ficoll PM400 density mean that isolated cells are found in sharp bands at the interface between layers of different density. This allows for easy removal of the purified sample with a pipette.

Second, cells with great differences in density can easily be isolated with as few as two density layers. This is achieved by choosing densities that will prevent one or more type of cell from entering the lower phase, banding these cell types at the interface. To estimate the densities required for a particular application, refer to Table 1.

Discontinuous gradients are established as follows:

1. Using Table 1 as a guide, dissolve Ficoll PM400 in buffer or isotonic (0.25 M) sucrose solution at various concentrations (generally differing by 5 to 10% w/v), which should separate the cells of interest. Most cells and organelles have a buoyant density between 1.0 and 1.2 g/ml in Ficoll PM400. Often, a simple two-layer gradient is sufficient. You may store these fractions in a refrigerator, but ensure that they reach room temperature before use.
2. In normal centrifuge tubes, make layers (approx. 1-cm deep) of decreasing density with the most dense solution at the bottom.
3. Keep the tubes at room temperature for a few hours to allow diffusion across the interfaces, and thereby even out the sharp borders between fractions.
4. Layer the suspension to be fractionated carefully on top. Stir the sample and upper Ficoll product layer gently with a glass rod to eliminate the interface between them before centrifugation.

During centrifugation, particles collect either in or between the various Ficoll product layers, depending on the density of the layers. The cells/organelles collect at a lower density than on sucrose gradients of equivalent concentration, as Ficoll product does not penetrate cell membranes. After centrifugation, pipette off the various phases, and remove the Ficoll product from the required fraction by repeatedly diluting with buffer, and centrifuging to sediment the particles. Residual amounts of Ficoll PM400 in the sample can be estimated with the anthrone reaction (1).

As an alternative to discontinuous gradients, you can easily prepare a continuous or linear density gradient of Ficoll product using a gradient mixer (13). In simple cases, you may only need one homogeneous solution (i.e., no density gradient). You can then fractionate by increasing centrifugation speed in steps. Ficoll PM400 has also been employed in zonal centrifugation studies (14).

Unit gravity sedimentation through a density gradient is widely used to separate cells intolerant to centrifugation (15).

Ficoll PM400 provides an economical alternative to albumin in such applications. Cells with similar densities but different size can also be efficiently separated at unit gravity (16, 17, 18). Ficoll PM70 has also been used, giving gradients of suitable osmolality yet very low viscosity (9).

Cell isolation

Isolation and purification of various cell types, organelles, protoplasts, cytoplasts, liposomes, minicells, bacterial cells and viruses can be accomplished on gradients of Ficoll PM400 (19, 20, 21, 22, 23).

Table 1. Examples of cells, viruses and subcellular particles separated in Ficoll PM400^a.

Source	Density ^b	Conditions
Membranes	1.05	100 000 × g for 16 h
Chromatophores	1.07	195 000 × g for 36 h
Brain vesicles	—	21 000 × g for 15 min
Hepatocyte cells	1.10-1.15	6000 × g for 2 h
Fibroblast cells	1.05	8000 × g for 60 min
Ehrlich ascites cells	1.07	1400 × g for 45 min

a = Data adapted from *Centrifugation: a practical approach*, D. Rickwood (ed.), IRLpress (Oxford and Washington D.C.) (1984).

b = Density is given as buoyant density.

Chemically defined cell culture media

Ficoll PM400 is used with and without serum-derived growth factors to support the growth of both primary cultures and established cell lines (24, 25).

Concentration dialysis

Ficoll PM400 is useful for concentrating solutions by dialysis since its high molecular weight and low content of dialyzable material prevents it crossing the dialysis membrane. Osmotic pressure draws water across the membrane into the solution of Ficoll PM400, effectively concentrating sensitive materials.

Electrophoresis

Continuous flow electrophoresis usually requires a stabilizer in the electrolyte. Ficoll PM400 is often employed in this capacity (26, 27).

Immunological studies

Ficoll PM400 can be employed as a hapten carrier, and has been conjugated to DNP, TNP, and FITC for the purpose of enhancing the primary immune response in mice. Conjugates with a range of substitution levels and minimal toxicity are easily prepared (28, 29, 30, 31).

Lymphocyte isolation

For routine separation of lymphocytes, we recommend Ficoll-Paque™ PLUS or Ficoll-Paque PREMIUM (32, 33). These are sterile, ready to use media of density 1.077 g/ml; they contain Ficoll PM400 (5.7% w/v) and sodium diatrizoate (9.0% w/v).

Lysis and cell particle isolation

Isotonic solutions and density gradients of Ficoll products are widely used to lyse protoplasts, and extract or isolate cell particles (19, 20, 21, 22, 23).

Molecular stabilization

Ficoll PM400 has been used as a cryoprotective agent for unstable biomacromolecules, as well as its cryoprotective properties, Ficoll PM400 has also been found to greatly contribute to the stabilization of sensitive macromolecules both in solution and during vacuum drying.

Nucleic acid hybridization

A common application has been the use of Ficoll PM400 in Northern and Southern blot analysis. Ficoll PM400 (0.02%), as a constituent in Denhardt's solution, reduces non-specific binding

of material to nitrocellulose membranes during nucleic acid hybridization (34, 35, 36).

Phase partitioning

Phase partitioning separates cells on the basis of surface properties. Ficoll PM400 is combined with polyethylene glycol in two-phase systems, and with dextran and polyethylene glycol in three-phase systems (37, 38).

Protein quantitation

An improved Ficoll PM400 density gradient method has been described for the correct determination of the protein content of crystals (39).

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Purification/isolation of:

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CY17816-22Dec20-DF

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Ficoll PM70 and Ficoll PM400 are supplied as dry powders in the following pack sizes:

Ordering information

Product	Pack size	Code no.
Ficoll PM70	100 g	17-0310-10
	500 g	17-0310-50
	5 kg	17-0310-05
Ficoll PM400	100 g	17-0300-10
	500 g	17-0300-50
	5 kg	17-0300-05
	40 kg	17-0300-08

Related products

Ficoll-Paque PLUS	6 × 100 mL	17-1440-02
	6 × 500 mL	17-1440-03
Ficoll-Paque PREMIUM	6 × 100 mL	17-1442-02
	6 × 500 mL	17-1442-03
Percoll™ PLUS	250 mL	17-1445-02
	1 L	17-1445-01
Percoll	250 mL	17-0891-02
	1 L	17-0891-01

