Cytodex 1 Gamma and **Cytodex** 3 Gamma

CELL CULTURE

Cytodex™ 1 Gamma and Cytodex 3 Gamma belong to Cytiva's Cytodex family of dextran-based surface microcarriers for cell culture applications. Cytodex products have a sponge-like microporous structure, which allows molecules of M_r ~ 100 000 in size enter the beads. Their bead size and density are optimized to support high cell growth rate and yield. The biologically inert polysaccharide matrix provides a stable, but non-rigid, tissue-like substrate for stirred cultures, from which cells are easily harvested. Cytodex microcarriers are transparent, allowing easy microscopic examination of attached cells.

In contrast to the standard Cytodex products, Cytodex Gamma microcarriers are delivered gamma sterilized. In addition, Cytodex Gamma products are supplied dry and shrunken to save storage space and facilitate transportation. To facilitate transfer to various cell culture vessels, the container is equipped with flexible connection options (Fig 1).

Key benefits of the Cytodex Gamma microcarriers include

- Sterilized and ready to use for quick culture start-up
- Supplied dry to save storage space and facilitate transportation
- Delivered in a convenient, single-use container system for easy transfer to various cell culture vessels
- Enable culturing of anchorage-dependent cells in single-use bioreactor to eliminate the need for cleaning and cleaning validation

Fig 1. Sterilized Cytodex Gamma microcarriers are supplied dry in polyethylene terephthalate (PET) containers with flexible connection options for various cell culture vessels.

Overview

The microcarrier concept was first conceived in 1967 by Professor van Wezel at the National Institute for Public Health and the Environment (RIVM), the Netherlands (1). In the first experiments, van Wezel used DEAE Sephadex™ A-50 ion exchange chromatography medium (resin) as a microcarrier. This strategy proved beneficial in initial experiments, as it provided a charged culture surface with a large surface area-to-volume ratio, a beaded form, good optical properties, and a suitable density. Glass spheres were not suitable, as their high density required stirring speeds that were not compatible with cell growth. Cytiva's Cytodex products are all based on the low-density Sephadex dextran bead that enables easy mixing and low shear.

As for a plastic culture surface, the transparent nature of Cytodex microcarriers allows studying cellular morphology as well as following culture progress and viral infections. Compared with cultivation on a plain plastic surface, however, the increased surface-to-volume ratio of microcarrier cultures enhances the volumetric productivity, while reducing the footprint of the manufacturing equipment. The high surface-to-volume ratio also allows waste disposal volumes to be greatly reduced. The microporous microcarrier structure enables formation of confluent cell layers around the bead with tight junctions to allow cells to secrete molecules into the interior of the beads though the basolateral side of the cell.

Thus, a different environment is generated inside the beads as compared with the surrounding cell culture medium, making it possible for the cells to communicate with each other through the bead under tissue-like conditions. Microcarrier cultures also allow part of the culture to be sampled for cell quality control or identification, while monitoring cell morphology and distribution in the remaining culture. As microcarrier concentration can be varied freely, from 1 g/L up to 25 g/L, the bioreactor output can be adjusted with regard to volumetric productivity. For high cell densities, culturing can be performed in perfusion mode in a bioreactor equipped with a cell retention filter.

Specifications

The main differences between standard Cytodex products and the Cytodex Gamma products are that Cytodex Gamma products are supplied shrunken and dried using ethanol, sterilized by gamma irradiation, and ready to use. Remaining ethanol (8% to 12%) inside the bead during gamma irradiation works as a quencher of free radicals during the sterilization process (2). Regardless of the remaining moisture, the Cytodex Gamma products are free-flowing powders, which make transfer to culture vessel easy. Compared with plastic solutions, the shrunken format (1.3 mL/g dry weight) of Cytodex Gamma microcarriers reduces volume during transport as well as storage space and associated cost. Upon use, Cytodex 1 Gamma and Cytodex 3 Gamma should be swelled to their full volumes of 14 and 18 mL/g dry weight, respectively.

Cytodex 1 Gamma and Cytodex 3 Gamma are validated sterile at a sterility assurance level (SAL) of 10^{-6} and delivered in single-use container systems. To facilitate transfer of the microcarriers to the culture vessel, the containers are equipped with flexible connection options. For aseptic transfer, the container can be connected to the bioreactor through a welding connection. Transfer is performed at a slight overpressure of dry air or inert gas to be applied via a sterile vent filter. Transfer of the free flowing microcarriers should always be performed dry. To save time and associated costs, there is no need for further sterilization or sterilization validation before use. The available validation support file, including safety data from studies of extractable and leachable compounds, facilitates regulatory filing.

The products are supplied in convenient package sizes for laboratory, pilot, and manufacturing scales corresponding to working volumes of 10, 100, and 1000 L based on a standard Cytodex concentration of 3 g/L. To avoid material waste, each container is designed to allow connection and reconnection up to four times. As the container systems can be linked in series, microcarriers from different containers can be pooled before use to bring flexibility to material consumption. Although the product is delivered sterile, the user should ensure further sterility by proper container handling during connection, transfer, and closure of the container.

Structure

Cytodex 1 Gamma is a general-purpose microcarrier designed by substituting a cross-linked dextran matrix with positively charged N, N-diethylaminoethyl (DEAE) groups distributed throughout the matrix. This product is particularly suitable for most established cell lines and for production of viruses or cell products from cultures of primary cells and normal diploid cell strains.

Cytodex 3 Gamma is designed by chemically coupling of a thin layer of denatured porcine collagen type 1 (gelatin) to the cross-linked dextran matrix. This product is the microcarrier of choice for cells that are difficult to culture *in vitro* and particularly for cells with an epithelial-like morphology. As the collagen surface layer can be digested by a variety of proteolytic enzymes, harvest of such cells from the microcarriers can be performed with maintained high cell viability and membrane integrity. This feature of Cytodex 3 Gamma is beneficial in the development of successful serial subcultivation protocols for scaling up culture volumes.

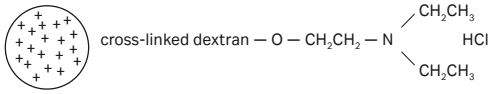
Before use, the gelatin is extensively processed:

- Subcontractor
  - Acid extraction
  - Filtration
  - Ion exchange chromatography
  - Ultrafiltration
  - Filtration
  - Steam sterilization
  - Drying
- Cytiva
  - 0.5 M Hydroxide treatment
  - Cross-linking
  - Drying
  - Gamma irradiation

Figure 2 gives a schematic view of the two types of Cytodex Gamma products. The physical characteristics of these microcarriers are shown in Table 1.

Cytodex 1

Positively charged groups
throughout the matrix



Cytodex 3

Collagen layer coupled to bead surface

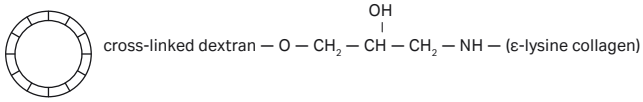


Fig 2. Schematic view of the two types of Cytodex Gamma microcarriers.

Table 1. Physical characteristics of Cytodex microcarriers

	Cytodex 1	Cytodex 3
Density* (g/mL)	1.03	1.04
Size*		
D_{50} (µm)	190	175
$D_{5–95}$ (µm)	147–248	141–211
Approx. area* (cm^2/g dry weight)	4400	2700
Approx. number of microcarriers/g dry weight	4.3×10^6	3.0×10^6
Swelling factor* (mL/g dry weight)	20	15

* In 0.9% NaCl. Size is based on diameter at 50% of the volume of a sample of microcarriers (d_{50}), or the range between the diameter at 5% and 95% of the volume of a sample of microcarriers ($d_{5–95}$). Thus, size is calculated from cumulative volume distributions.

Container systems

Cytodex Gamma products are packaged into container systems specifically designed for flexible transfer to the culture vessel (Fig 3). The containers can withstand pressures from 0.3 to 0.4 bar during transfer. Solid containers are used to minimize the risk of grinding of the dry powder during transport and to prevent the generation of extractables and leachables. Container system and packaging components are listed in Table 2.

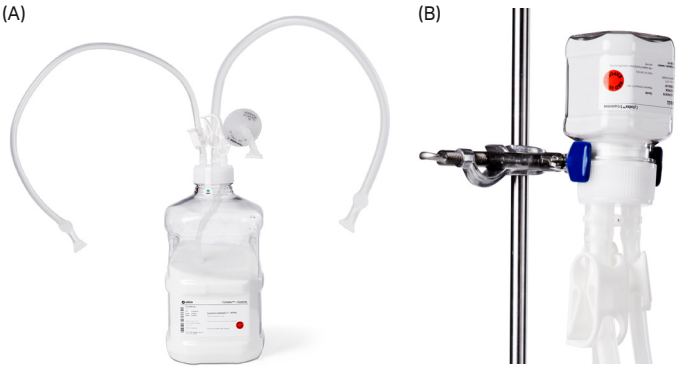


Fig 3. (A) The containers are equipped with a special cap and tube assembly to facilitate transfer and flexibility in connectivity. Each container can be connected up to four times: twice through welding using two tubes of different sizes. (B) The containers are aseptically connected to the culture vessel, pressurized, and turned upside down for transfer. Overfilling is performed to compensate for any material hold-up.

Table 2. Container system and packaging components

Container system components	Material	Max. gamma dose (kGy)
Bottle	Polyethylene terephthalate (PET)	1000
Ported cap	High-density polyethylene (HDPE)	1000
Nylon strap	Nylon 6,6	45 to 50
Transfer tube	C-Flex™ thermoplastic elastomer (TPE)	40
Vent/dip tube	Platinum silicone	50
Midisart™ BV 17805 sterilizing filter	Polyethylene fluoroethylene (PTFE) membrane + polypropylene (PP) housing	50
Pinch clamps	Polyester	50
Package components	**Material**	**Max. gamma dose (kGy)**
Protecting plastic film	Low-density polyethylene (LDPE)	50
Cardboard box	Cellulose	100 to 200

Quality control testing

Performance of Cytodex 1 Gamma and Cytodex 3 Gamma is validated with serum free medium. Every batch conforms to stringent specifications. Quality control tests are performed to satisfy both physiochemical and functional (i.e., cell growth) properties. A certificate of analysis is available from www.cytiva.com/certificates.

Bio safety

All polymeric materials of the container systems comply with United States Pharmacopeia (USP) <88> Biological Reactivity Test Class VI and are free from animal-derived components in compliance with EMA/410/01.

ISO certification

Cytodex 1 Gamma and Cytodex 3 Gamma products are manufactured in compliance with Cytiva's ISO 9001 certified quality management system and filled in an ISO 14644:1999 class 8 environment.

Viral and BSE inactivation

Cytodex 1 Gamma is animal origin free.

Cytodex 3 Gamma contains pig skin gelatin. The gelatin is heat-treated during manufacture.

The heating step inactivates all viruses investigated. The risk for a naturally BSE infected pig is virtually none (see virus risk assessment supplied with the product's Validation file).

Furthermore Cytodex Gamma beads are treated with 0.5 M NaOH during synthesis. After crosslinking, the beads are irradiated with >30 kGy gamma irradiation. These treatments have been shown to be effective in inactivating BSE and in eliminating viruses (3).

Cytodex 1 Gamma is 0.5 M NaOH treated and crosslinked twice.

Cytodex 3 Gamma is 0.5 M NaOH treated and crosslinked three times. One of these is after gelatin addition.

Shelf life

A two-year forced shelf life study was performed according to the ASTM F1980 guideline at 50°C in ambient humidity (10% to 50% relative humidity [RH]). As the Cytodex Gamma products are hygroscopic, the accelerated study was complemented with a stress test at 40°C in 75% RH. The studies are available as part of the validation guide available from www.cytiva.com/rsf.

The shelf life of Cytodex 1 Gamma and Cytodex 3 Gamma is two years.

Extractable and leachable compounds

A study of extractable and leachable compounds is available as part of the validation guide available from www.cytiva.com/rsf.

Sterilization

Gamma irradiated Cytodex 1 Gamma and Cytodex 3 Gamma products are validated sterile at a SAL of 10^{-6} according to ISO/AAMI/ASTM 11137.

Security of supply

Cytodex 1 Gamma and Cytodex 3 Gamma products are manufactured in Uppsala, Sweden. The manufacturing site is open for and regularly subjected to customer audits.

Cytodex 1 Gamma and Cytodex 3 Gamma are supported by our security of supply services. To facilitate regulatory filing, validation support and regulatory support files are available upon request.

Applications

Microcarrier-based culturing can be performed in simple shaker flasks or roller bottles as well as in more advanced traditional or single-use bioreactor systems, from milliliter to thousands-of-liter scales. Compared with cultivation in several shake flasks or roller bottles, one single bioreactor culture allows:

- Automatic control of pH and dissolved oxygen (DO), in-line cell counting, and logging of cell culture data for product documentation
- Perfusion culturing to high cells densities, minimizing the need for several intermediate seed train bioreactors during scale-up
- Scaling to be performed in one bioreactor system
- Inactivation of harmful organisms in the entire culture batch to be performed in just one step

Microcarrier retention system

By connecting or integrating a microcarrier retention system (a plastic or stainless steel mesh of 80 to 100 µm) into the bioreactor, several process steps can be performed while keeping the culture contained in the culture vessel. The use of a microcarrier retention filter allows:

- Wash of the microcarrier culture with cell culture medium
- Detachment and separation of cells from microcarriers during scaling
- Concentration of the microcarrier culture for medium exchange prior to viral infection or cell lysis (non-lytic viruses)
- Separation and harvest of lytic viruses from microcarriers with remaining cell debris
- Perfusion culturing for high cell densities to increase volumetric productivity, while keeping a small equipment footprint

Critical aspects of microcarrier technology

Microcarrier cultures comprise a large number of small colonies, with each bead being a separate colony. The most critical step of microcarrier cultures is the inoculation step. It is important to inoculate with a sufficient amount of viable cells for each colony to become confluent for full utilization of the microcarrier surface area. Cells that do detach during mitosis migrate and colonize other beads to even out an uneven inoculation. As cell aggregates can generate bead-bridging, causing aggregate settling and heterogeneous culture, it is important that a true single-cell suspension is used for inoculation.

When scaling up the culture process, the lowest possible inoculation density should be established for the specific cell line, cell culture medium, and microcarrier concentration to be used. The larger the split ratio, the lower the number of subcultivation steps and bioreactor scales required in seed train culturing. In general, the richer the cell culture medium, the lower the possible inoculation density and the larger the split ratio.

For microcarrier-to-microcarrier subcultivation, it is necessary to consider the high cell concentration attained upon microcarrier sedimentation. The swollen volume of Cytodex 1 Gamma is 20 mL/g dry weight. In a 1 L culture using 3 g Cytodex 1 Gamma (= 60 mL), for example, a typical cell density achieved can be ~ 1×10^9 cells/L depending on cell type and culture medium used. Upon microcarrier sedimentation, the cells will be concentrated down into 60 mL, giving a cell concentration of ~ 2×10^7 cells/mL. For an efficient cell harvest, a 10-fold concentration of cell detachment enzyme to what is normally used in cell harvest from a plastic surface should be used for the cell-to-enzyme ratio to be the same. For enhanced cell detachment, a mixture of different cell detachment enzymes that cut in different protein sequences can be used.

References

1. van Wezel, A. L. Growth of cell strains and primary cells on microcarriers in homogeneous culture. *Nature* **216**, 64–65 (1967).
2. Henderson, A. M. and Rudin, A. ESR study of the effects of water, methanol, and ethanol on gamma-irradiation of starch. *Journal of Polymer Sciences: Polymer Chemistry Edition.* **19**, 1721–1732 (1981).
3. Instructions: Cytodex 1 and Cytodex 3 Gamma microcarriers, Cytiva, 29115815, Edition AA (2016).

Ordering information

Product	Size	Product code
Cytodex 1 Gamma	30 g	17548701
	300 g	17548702
	3 kg	17548703
Cytodex 3 Gamma	30 g	17548801
	300 g	17548802
	3 kg	17548803

Cytodex Gamma products stored under dry conditions in unopened package are stable for more than two years. Cytodex Gamma products that have been hydrated and sterilized can be stored sterile in phosphate buffered saline (PBS) at 4°C for at least two years.

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