# **Cytodex** 1, Cytodex 3

## Cell culture

## Instructions for Use

Microcarrier culture is a technique which makes possible the practical high yield culture of anchorage-dependent cells. Cytodex™ has been specifically developed for the culture of a wide range of animal cells in culture volumes ranging from a few milliliters to more than six thousand liters. Using Cytodex in simple suspension culture systems provides yields of several million cells per milliliter. It also makes it easy to change the available surface area, simply by changing the microcarrier concentration.

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Read these instructions carefully before using the products.

**Intended use**

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

**Safety**

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

# 1 Introduction

## Applications

Cytodex introduces new possibilities for the culture of animal cells. The microcarriers provide convenient surfaces for the growth of animal cells and can be used in suspension culture systems, or can be used to increase the yield from monolayer culture vessels (e.g., wells, dishes, bottles) and perfusion chambers. Applications include the production of large quantities of cells, viruses and recombinant cell products (e.g., interferon, enzymes, nucleic acids, hormones), studies on cell adhesion, differentiation and cell function, perfusion column culture systems, microscopy studies, harvesting mitotic cells, isolation of cells, membrane studies, storage and transport of cells, assays involving cell transfer and studies on uptake of labelled compounds.

## Properties

Cytodex is designed to meet the special requirements of a microcarrier technique.

- The size and density are optimized to give good growth and high yields for a wide varity of cells.
- The matrix is biologically inert and provides a strong but nonrigid substrate for stirred microcarrier cultures.
- The microcarriers are transparent, allowing easy microscopic examination of the attached cells.

Cytodex 1 is based on a cross-linked dextran matrix which is substituted with positively charged N, N-diethylaminoethyl groups. The charged groups are distributed throughout the microcarrier matrix and the resin has an ionic capacity of 1.4 to 1.6 mmol $Cl^-$/g of resin.

Cytodex 3 consists of a thin layer of denatured collagen chemically coupled to a matrix of cross-linked dextran. The denatured collagen layer on Cytodex 3 is susceptible to digestion by a variety of proteases, including trypsin and collagenase, and provides unique opportunities for removing cells from the microcarriers while maintaining maximum cell viability, function, and integrity.

## Characteristics of Cytodex

| | Cytodex 1 | Cytodex 3 |
|---|---|---|
| Density[1] (g/mL) | 1.03 g/mL | 1.04 g/mL |
| Particle size, $d_{50v}$ [1, 2, 3] | ~170 µm | ~150 µm |
| Particle size distribution[1, 2, 4] | 140-200 µm | 120-180 µm |
| Approx. area (wet)[1, 2] | 3000 $cm^2/g$ | 2000 $cm^2/g$ |
| Approx. no. microcarriers/g dry weight[2] | $4 \times 10^6$ | $3 \times 10^6$ |
| Swelling factor[1, 2] | 20-25 mL/g dry weight | 15-18 mL/g dry weight |
| Sedimentation velocity[2, 5] | 90 cm/h | 120 cm/h |

1 In 0.15 M NaCl solution.

2 Some batch-to-batch variations may exist.

3 Median particle size of the cumulative volume distribution.

4 ≥ 70% volume share within given range.

5 Measured in a 64 x 370 mm measuring cylinder using 3 g/L in 0.15 M NaCl solution. Values measured on empty microcarriers without cells.

## Which Cytodex to use?

Full details for selecting the correct type of Cytodex for a given application can be found in the book *Microcarrier Cell Culture: Principles and Methods* (18114062) which is avaible from Cytiva.

Cytodex 1 is suitable for general purpose microcarrier culture, particularly for most established cell lines. This microcarrier can also be used for production from cultures of primary cells and normal diploid cell strains when maximum recovery of culture products is not essential.

Cytodex 3 is the microcarrier of choice for cells known to be difficult to grow in culture, for differentiated cell culture systems, and particularly for cells with an epithelial-like morphology. Furthermore, this microcarrier is recommended for scaling up and for when simplified harvesting is required with maximum retention of cell viability and membrane integrity.

***Note:*** *Cytodex 3 can be used as a general purpose collagen-coated culture surface.*

# 2 Operation

## Preparation

| Step | Action |
|---|---|
| **1** | Hydrate the microcarriers in $Ca^{2+}$ and $Mg^{2+}$ free PBS (50 to 100 mL/g of Cytodex) for at least 3 h at room temperature.<br><br>***Note:***<br>*When hydrating Cytodex 3, initial surface tension may occasionally prevent wetting and sedimentation of the microcarriers. Should this occur, Tween 80 can be added to the PBS used for the first hydration rinse (2 to 3 drops Tween 80/100 mL PBS).* |

| Step | Action |
|---|---|
| **2** | Decant the supernatant and wash the microcarriers in fresh $Ca^{2+}$ and $Mg^{2+}$ free PBS (30 to 50 mL/g of Cytodex) for 2 to 3 minutes. |
| **3** | Discard the PBS and replace with fresh $Ca^{2+}$ and $Mg^{2+}$ free PBS (30 to 50 mL/g of Cytodex). |
| **4** | Sterilize the microcarriers in an autoclave at 115°C, 15 psi for 15 minutes.<br>***Note:***<br>*Cytodex is extremely stable and can be autoclaved repeatedly (at least 5 cycles) or extensively (130°C, 12 h, 27 psi) without affecting the performance. The pH of all solutions should be 7.4.* |
| **5** | Prior to use, allow the microcarriers to settle and remove the supernatant. |
| **6** | Rinse the microcarriers briefly in warm culture medium (20 to 50 mL/g of Cytodex). |
| **7** | When the microcarriers have settled, discard the supernatant and transfer them to the culture vessel. |

## Culture vessels

Full details on culture vessels can be found in *Microcarrier Cell Culture: Principles and Methods*.

In brief, microcarrier cultures can be contained in virtually any type of cell culture vessel. However, the best results are obtained when using equipment that gives even suspension of the microcarriers with gentle stirring. The most suitable vessels for general purpose microcarrier culture are those which result in a homogeneous culture environment due to an efficient mixing system that does not generate high shear forces. In selecting a vessel, some design criteria must be considered. It is important that the stirrer should never come into contact with the inside surface of the vessel during culture since this may damage the microcarriers. Similarly, spinner vessels having a bearing which is immersed in the culture medium are not suitable since the microcarriers can circulate through the bearing and become crushed. A variety of compatible design systems are commercially available, the choice of which is essentially dependent upon the size of culture vessel required. Alternatives exist for laboratory, pilot, and production scale applications. Contact Cytiva for more information.

***Note:*** *Glass culture vessels should be siliconized before use.*

## Culture procedure

The exact culture procedure depends on the type of cell and the culture vessel. Culture procedures are described in detail in *Microcarrier Cell Culture: Principles and Methods*. Microcarrier cultures normally contain 1 to 5 g of Cytodex/L, are inoculated with $5 \times 10^4$ to $2 \times 10^5$ cells/mL, and are stirred at 20 to 60 rpm, depending upon the design of the system. Perfused microcarrier cultures can contain up to 20 g of Cytodex/L.

A useful procedure when first starting with suspension microcarrier cultures is as follows (volumes and the inoculation size can be adjusted proportionately for different sized cultures).

| Step | Action |
|---|---|
| **1** | For 100 mL of culture, add 0.3 g of Cytodex to the spinner vessel in 30 mL of culture medium. |
| **2** | Innoculate the culture with $10^7$ cells, mix gently, and incubate at 37°C. |
| **3** | Commence continuous stirring once the cells have firmly attached to the surface of the microcarriers.<br>***Note:***<br>*The time period required for this attachment is primarily dependent upon the attachment efficiency of the cell type. If the time for attachment is exessive, it is desirable to stir the culture intermittently (e.g., for 2 min every 30 min) to ensure an even distribution of the cells and microcarriers.* |
| **4** | Once the microcarriers are evenly distributed, increase the culture volume to 50 mL.<br>***Note:***<br>*The speed of stirring depends on the culture vessel and should be sufficient to prevent sedimentation of the microcarriers.* |
| **5** | After 1 to 2 days, increase the culture volume to 100 mL. A partial change of medium may be required after 3 to 5 days. |

***Note:*** *Modifications to this procedure may be necessary for each type of cell.*

## Monitoring cell growth

Representative samples of the microcarriers can be withdrawn from the culture and examined microscopically, either directly with phase contrast or after staining with, for example, haematoxylin. The most suitable method for determining cell number is to use the standard nucleus extrusion method (Sanford, K.K., Earle, W.R., Evans, V.J. et al., J. Nat. Cancer Inst. 11 (1951) 773–795).

## Harvesting cells

Various methods can be used to remove cells from Cytodex. The most common method is to use standard procedures with proteolytic enzymes such as trypsin. Collagenase can be used for Cytodex 3.

| Step | Action |
|---|---|
| **1** | Allow the microcarriers to settle and remove the culture medium. |
| **2** | Wash the microcarriers for 5 min in $Ca^{2+}$ and $Mg^{2+}$ free PBS containing 0.02% (w/v) ethylenediaminetetraacetic acid (EDTA), pH 7.6. (50 to 100 mL/g of Cytodex).<br>***Note:***<br>*At higher serum concentrations, more extensive washing might be required. Contact Cytiva for more information.* |

| Step | Action |
|---|---|
| **3** | Remove the EDTA-PBS and replace with either trypsin or collagenase solution (approx. 30 to 50 mL/g of Cytodex). Mix well and incubate at 37°C with occasional agitation. |
| **4** | After 15 minutes, stop the reaction by adding culture medium containing serum (20 to 30 mL medium/g of Cytodex).<br>***Note:***<br>*Any cells remaining in the microcarriers at this stage can be removed by gentle agitation.* |
| **5** | The detached cells can be separated from the microcarriers by sedimentation at unit gravity (for routine harvesting) or by filtering through a 100 µm filter (for maximum recovery) and used for the inoculation of a subsequent microcarrier culture (e.g., during scaling up of a production process). |

***Note:*** *If satisfactory cell yields are not obtained by following the above outlined EDTA-trypsin procedure, the process can be optimized by more careful consideration and standardization of the activity of the trypsin solution. Contact Cytiva for more information.*

***Note:*** *When harvesting cells from Cytodex for use as an inoculum in a subsequent microcarrier culture, it is necessary to obtain a suspension of single cells with maximum viability, retained membrane integrity and high attachment efficiency. Therefore, the speed of washing and harvesting is very important.*

In order to obtain maximum cell recovery after harvesting, it is also possible to detach the cells from Cytodex with dextranase. The enzyme activity will digest the dextran-based matrix of Cytodex and result in a free cell suspension after about 15 min exposure to the enzyme solution.

## Quality control

Each batch of Cytodex is subjected to stringent tests to examine both physiochemical and functional properties. One cell type is cultured over a week-long period to ensure the microcarriers support high density cell growth. A Certificate of Analysis containing the results of these tests is available on request for all batches of Cytodex

## Availability and storage

Cytodex is supplied as a dry powder and must be hydrated and sterilized before use. The following pack sizes are available:

| | 10 g | 25 g | 100 g | 500 g | 5000 g |
|---|---|---|---|---|---|
| Cytodex 1 | - | 17044801 | 17044802 | 17044803 | 17044804 |
| Cytodex 3 | 17048501 | - | 17048502 | 17048503 | 17048504 |

Packs of Cytodex stored unopened under dry conditions are stable for more than eight years. Alternatively, Cytodex which has been hydrated and sterilized as described above, can also be stored sterile in PBS for at least two years at 4°C.

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