Cytodex 1 and Cytodex 3 Gamma microcarriers

Instructions for Use

cytiva.com

Table of Contents

1 Introduction

About this manual

These *Instructions* provide the information needed to use Cytodex™ 1 and Cytodex 3 Gamma microcarriers in a safe way.

In this chapter

1.1 Important user information

Intended use of Cytodex 1 and Cytodex 3 Gamma microcarriers

Cytodex Gamma container system is intended for transfer of the Cytodex Gamma microcarriers into bioreactors, mixers, or other Cytodex Gamma container systems.

These gamma sterilized products can be used to support various adherent cell culture processes and minimize microcarrier handling procedures (hydration, sterilization, and washing).

Prerequisites

- The recipient bioreactor, mixer or bag must be equipped with the corresponding C-Flex® tubing.

  Note: *Other connectivity design can only be used after validation of clogging-free transfer. This is not a Cytiva responsibility.*

  Note: *The recipient tubing must not be moist since this increases the risk of clogging during transfer.*

- The prerequisites for dosing are described in *Chapter 5 Dosing, on page 45*.

Safety notices

This user documentation contains safety notices (WARNING, CAUTION, and NOTICE) concerning the safe use of the product. See definitions below.

WARNING

WARNING indicates a hazardous situation which, if not avoided, could result in death or serious injury. It is important not to proceed until all stated conditions are met and clearly understood.

CAUTION

CAUTION indicates a hazardous situation which, if not avoided, could result in minor or moderate injury. It is important not to proceed until all stated conditions are met and clearly understood.

NOTICE

NOTICE indicates instructions that must be followed to avoid damage to the product or other equipment.

1.2 Regulatory information

Materials

All the materials used for the packaging, directly in contact with sterile Cytodex Gamma microcarriers, meet USP class VI <class 88> requirements (biological test for plastics). All the materials used for the product, including Cytodex Gamma microcarriers withstand a maximum gamma irradiation dose of 40 kGy.

The material cannot be resterilized by further gamma irradiation or by autoclaving.

Sterility

The microcarrier powder pathway is validated sterile at a SAL 10^{-6} according to AAMI TIR 33:2005 and the principles of ISO/AAMI/ASTM 11137-1:2006, VD 27.5. Sterility validation data is part of the *Validation Guide*.

The containers are delivered sterile as single use products. As soon as the packaging has been opened, it is the user's responsibility to uphold the product sterility by proper container handling during connection, transfer and closure.

Biosafety

The container system complies with United States Pharmacopea (USP) <88> Biological Reactivity Test Classs VI and are free from animal derived ingredients in compliance with EMA/410/01.

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 steps inactivates all viruses investigated. The risk for a naturally BSE infected pig is virtually none. See reference 20030604.

Furthermore Cytodex Gamma beads are treated with 0.5 M NaOH during synthesis. After crosslinking, the beads are irradiated with >30 kGray Gamma irradiation. These treatments have been shown to be effective in inactivating BSE and in eliminating viruses. For more information see Application note 18112457.

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.

ISO

This product has been filled in an ISO 14644:1999 class 8 environment and manufactured in compliance with Cytiva ISO 9001 certified quality management system.

Shelf life

A two-year accelerated shelf life study was performed according to the ASTM F1980 guideline, at 50°C in ambient humidity (10-50%RH) . As the Cytodex Gamma microcarriers are hygroscopic the accelerated study was complemented with a stress test at 40°C 75% RH.

The studies are available as part of the *Validation Guide*.

The differences between Cytodex Gamma and Cytodex

Cytodex and Cytodex Gamma are not identical products.

Acetone is used to shrink and dry Cytodex and ethanol is used to shrink and dry Cytodex Gamma . Cytodex is sterilized by autoclaving and Cytodex Gamma is sterilized by gamma irradiation.

Certificates of Quality

Certificates of Quality are available at *cytiva.com/certificates*.

Validation Guides are available at *cytiva.com/rsf*.

Extractables and leachables

An *Extractables and Leachables* study is available as part of the *Validation Guide*.

1.3 Recommendations for use

Precautions

WARNING

Do not use the product in any other way than described in these instructions.

WARNING

The container system must NOT be used for any other purposes than transfer of Cytodex Gamma microcarriers. The containers are NOT pressure vessels!

CAUTION

There is not sufficient space to swell the microcarriers inside the containers.

CAUTION

The container system cannot be re-sterilized by gamma irradiation or autoclaving.

CAUTION

Do not use containers if their packaging is opened or damaged.

NOTICE

The use of connectors other than ReadyMate™ connectors that are 9.5 mm and larger is not recommended. Cytiva cannot guarantee that they will not block the transfer of Cytodex Gamma microcarriers.

NOTICE

Keep dry as the product is hygroscopic.

NOTICE

Protect against sunlight.

Recommendations for Cytodex Gamma container systems

The table below describes the important functions related to Cytodex Gamma container system .

Parts	Function
Cytodex Gamma container system	The container system and microcarriers should be used dry. Pressure applied to the container must not exceed 0.5 bar (7 psi). The recommended transfer pressure is 0.3 to 0.4 bar (4.4 to 5.6 psi).
Tubing	Always weld tubing of corresponding brand, material and dimensions to the container system transfer tubing and welding connections. Minimize the length of tubing to facilitate speed of transfer, without reducing the amount needed for possible subsequent connections. Avoid the use of long, narrow or moist tubing for transfer, as the microcarrier may swell and clog the transfer line. Three kilograms of Cytodex 3 Gamma can be transferred in < 5 minutes with wide tubing and a length of 1 m (25 + 75 cm).
Filter	The gas inlet to the container is equipped with an integrity tested Midisart™ BV 17805 sterile vent filter.
Gas	As the product is intended to be used dry, only a dry gas should be used. Use dry air or an inert gas (nitrogen). Use a manometer to make sure the correct transfer pressure is used.
Manual pressurization (Peleus or pipetting ball)	A Peleus or pipetting ball is a simple and convenient way to generate overpressure. However, it has to be used with caution to prevent pressures above 0.5 bar (7 psi). A pressure guage should be connected to monitor pressure.
Connectivity	As the product is provided sterile, it is of utmost importance to perform correct aseptic connections to maintain sterility. It is possible to connect each Cytodex Gamma container system at least four times by welding the two transfer tubes.

Parts	Function
Welding	Welds should be made with the same kind and dimension of thermoplastic elestomer tubing (C-Flex) to ensure aseptic connection. Use a calibrated/ validated weld instrument to guarantee weld integrity. To save remaining Cytodex material in the container and maintain sterility, seal off the connection after use.
Extractables and Leachables	Toxicon AB has performed interdependent studies that are included in the the *Validation Guide*. Cytiva has tested leachables from the transferred microcarriers in two different solvents. As the product is intended for use when dry, Cytiva has not evaluated these solvents on the packaging.
Seal	It is important to perform sealing correctly to maintain product sterility. Sealing off tubing containing Cytodex Gamma microcarriers is a potential risk. It is the user's responsibility to evaluate the integrity and strength of the seal. It is important to empty the tubing from microcarriers before attempting any sealing. An alternative to sealing is to clamp the tubing with validated clamps.

1.4 Associated documentation

More information

For more information regarding Cytodex Gamma microcarriers and their use, refer to the following documentation:

- *Cytodex 1 and Cytodex 3 Gamma Data file, article number 18158445*
- *Instructions 18111979*
- *Microcarrier cell culture, principles and methods, article number 18114062*
- *ReadyMate Disposable Aseptic Connectors, article number 28937902*
- *Disposable Cellbag™ bioreactors for WAVE Bioreactor™ systems, article number 28951136*
- *Xcellerex XDR cell culture bioreactor systems, article number 29092925*

Data files, application notes and user documentation on the web

To order or download data files, application notes or user documentation, see the instruction below.

Step	Action
1	Go to *cytiva.com*.
2	Select ***Cytodex 1 Gamma*** or ***Cytodex 3 Gamma***.
3	Select ***Documents***.
4	***Note:*** *If you wish to print these instructions, set print to page and standard format.*

2 Product description

In this chapter

2.1 General information

Introduction

Cytodex Gamma container system consists of Cytodex 1 Gamma or Cytodex 3 Gamma microcarriers, packaged in sterile, ready-to-use, gamma irradiated plastic container systems.

The dry Cytodex Gamma microcarriers can be transferred aseptically using low-pressure dry air, or inert gas, applied via a sterile vent filter into bioreactors, mixers, or other Cytodex Gamma container systems. Transfer of the free-flowing microcarriers must always be performed dry.

Cytodex Gamma container system can be connected aseptically at least four times by two connectivity options.

Pack sizes

Cytodex Gamma container system is available in three pack sizes: 30 g, 300 g and 3 kg, in 125, 500 and 5000 ml PET containers respectively.

Tubing

The Cytodex Gamma PET container is equipped with a ported cap having three external and one internal ports.

There are four tubes fitted to the ported cap:

- A sealed transfer tube.
- A second sealed transfer tube.
- A vent tube.

- A dip tube connected to the internal port inside the container.

The ported caps are closed with a torque of 8 to 10 Nm to ensure Cytodex Gamma container system integrity.

Vent filter and dip tubing

The vent and the dip tubing are made of platinum silicone. The vent filter is equipped with a protective tubing which should cover the vent filter during storage.

The dip tubing is long enough to stand clear of the settled microcarrier bed when the container system is turned upside down.

Pinch clamps

The Cytodex Gamma microcarriers flow can be stopped with pinch clamps or hemostatic forceps, during the transfer process.

System label

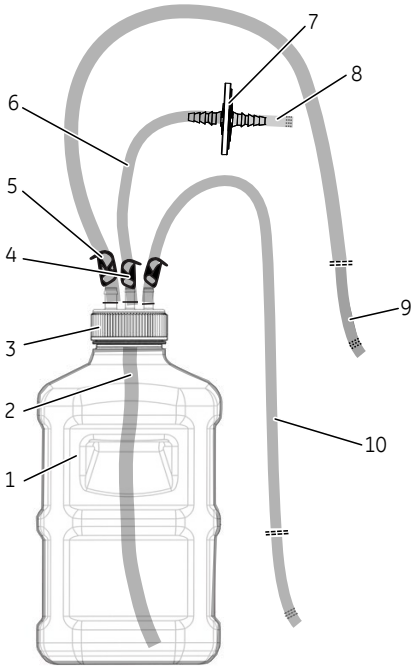
The system label contains the product name and other product information.

Label text	Description
Code number	Code number for the article.
Pack size	Pack sizes: 30 g, 300 g, and 3 kg.

Label text	Description
Lot	Lot number. The lot number ensures the Cytodex Gamma container system is fully traceable to the production source.
Pack	Packing date.
Expiry	Estimated expiry date.
Bar code	The bar code (GS1-128, a total of 30 digits) contains an application identifier1 (90), the code number (digits 3 to 10), a second application identifier (10), lot number (digits 13 to 20), a third application identifier (21) and the ID number (digits 23 to 30).
Sterile	The powder pathway is validated sterile at a SAL 10^{-6} according to the principles of ISO/AAMI/ASTM 11137.
Pressure	Maximum pressure 0.5 bar.
Instruction is available at	*cytiva.com* Select: ***Cytodex 1 Gamma*** or ***Cytodex 3 Gamma***. Select: ***Documents***.
Red is exposed 	Irradiation indicator. Red - Indicates that irradiation has been applied to the product. Orange - Indicates that the product has not been irradiated.

2.2 Dimensions

Container system



Item	Part		30 g	300 g	3 kg
1	PET container	Volume	125 ml	500 ml	5 L
		W × H × D	59 × 95 × 59 mm	83 × 153 × 83 mm	168 × 325 × 168 mm
2	Sani-Tech Ultra 50 dip tubing (ID × OD × L)		6.4 × 11.2 × 75 mm	6.4 × 11.2 × 130 mm	6.4 × 11.2 × 290 mm
3	Three-ported cap		49 mm	49 mm	70 mm
4	Pinch clamp × 2 (small)		Max tubing diameter 11.4 mm		
5	Pinch clamp (large)		Max tubing diameter 19 mm		

Item	Part	30 g	300 g	3 kg
6	Sani-Tech Ultra 50 vent tubing	6.4 × 11.2 × 150 mm		
7	Vent filter	Multiple step hose barb		
8	C-flex protective tubing (ID × OD × L)	6.4 × 11.2 × 60 mm		
9	C-flex transfer tubing (ID × OD × L)	9.5 × 15.9 × 750 mm		
10	C-flex transfer tubing (ID × OD × L)	6.4 × 11.2 × 750 mm		

2.3 Material description

Container components

Component	Supplier	Material	Max Gamma dose (kGy)
Container, (PharmaTainer™)	Cellon SA	PET (PolyEthyleneTerephtalate)	1000
Ported cap	Cellon SA	HDPE (HighDensityPolyEthylene)	1000
Nylon strap	Panduit	Nylon 6.6	45 to 50
Transfer tubing, C-flex	Saint-Gobain Performance Plastics	TPE C-flex 374	40
Vent/dip tubing	Saint-Gobain Performance Plastics	Platinum silicone	50
Sterilizing filter, Midisart BV 17805	Sartorius Stedim	Reinforced PTFE (PolyTetraFluoroEthylene) membrane and polypropylene housing	50
Pinch clamps	Halkey Roberts	Polyester	50

Packaging components

Component	Supplier	Material	Max Gamma dose (kGy)
Protective plastic bags	Polypac	LDPE (Low Density Polyethylene)	50
Cardboard box	Svensk Emballageteknik	Cellulose	100-200

2.4 Loss of material

NOTICE

Neither the containers nor Cytodex Gamma can be re-sterilized by autoclaving or gamma irradiation.

Pack size

The loss of material during transfer is listed below.

Pack size	Loss of material
30 g	3.2 to 4.6 g
300 g	4.8 to 6.2 g
3 kg	13.6 to 15 g[1]

[1] *Note:* Shorter transfer tubing eases transfer and reduces material loss.

C-flex tubing

The loss in g/cm tubing during connecting is listed below.

ID C-flex tubing	Loss of material
6.4 mm	0.01 g loss/cm
9.5 mm	0.02 g loss/cm

Filling range

The loss of material has been taken into account and the respective pack size is overfilled according to the table below.

Pack size	Filling range
30 g	35 to 38 g
300 g	307 to 315 g
3 kg	3015 to 3050 g

3 Installation

In this chapter

3.1 Unpacking

Outer packaging

The product is packed in an unsealed plastic bag inside a cardboard box.

Dust protection

Inside the first plastic bag are two sealed plastic bags that protect the product from dust.

Transport and handling protection

A protective pouch covers the tubing, clamps and cable ties on the 300 g and 3 kg packs. The pouch prevents puncture of the dust protection barriers. As long as the inner dust protection bag is intact, a container system is still functional even when the protective pouch is punctured.

Sterile barrier system

The Cytodex Gamma container system is the primary sterile barrier system. Cytodex Gamma container system includes the PET container and all add-on components (tubing, connectors, pinch clamps etc.).

Certificate of Analysis

A Cytiva Certificate of Analysis for the Cytodex Gamma microcarrier container is available at *cytiva.com/certificates*. In addition, manufacturing information, product release criteria, and regulatory conformance are available at this address.

Unpacking the Cytodex Gamma container system

Step	Action
1	Keep the cardboard box standing with the arrows pointing upwards to prevent Cytodex Gamma microcarriers pouring into the tubing and the vent filter.

2	Check the expiry date of the assembly. Make sure the gamma radiation label is red, indicating that the material has been irradiated.

Step	Action
3	Open the cardboard box and check the assembly for damage. Remove first unsealed bag, which is provided for packing, transportation and unpacking protection. Make sure the sealed outer and inner plastic bags are undamaged. Do not use if the packaging is opened or damaged.

Step	Action
4	Open the outer sealed plastic bag and transfer the material into the cleanroom area.

In the cleanroom

Step	Action
1	Open the inner plastic bag in the cleanroom just prior to use.

Step	Action
2	Remove the plastic pouch from the 300 g and 3 kg pack sizes.

Step	Action
3	Place the container system in upright position. For small container systems, use a beaker to hold it upright as they are top-heavy.
	
4	Remove the the cable ties that hold the tubing.
5	Empty all the tubing of any microcarriers by raising the tubing and shaking the material back into the container.
6	Check that all pinch clamps are located as close as possible to the outlets on the ported cap. Close all the pinch clamps to prevent microcarriers from pouring into the tubing and vent filter if the container is turned upside down.
7	Remove the protective tubing from the vent filter and save it for further use.
8	Bring the container(s) to the bioreactor, or vessel, to be loaded with Cytodex Gamma microcarriers.

3.2 Setup

Mounting the container system

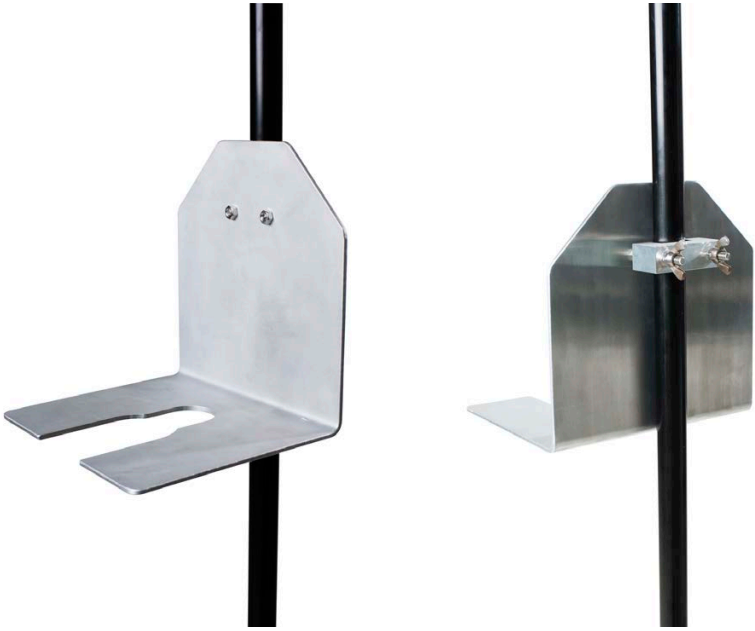
Mount the container system upside down during the transfer process.

The container system can be mounted with a standard clamp and lab stand. The specially designed holding plate can also be used, see description below.

Holding plate

The holding plate has two positions, one for the 42 mm bottleneck (30 g and 300 g pack sizes), and one for the 61 mm bottleneck (3 kg pack size). The height of the holding plate can be adjusted with the screws.

Note: *The holding plate is not available as a product from Cytiva. For dimensions of the holding plate, see* *Section 8.1 Holding plate design, on page 67**.*



Large bottleneck mounted on the holding plate

The larger bottleneck (diameter 61 mm) fits the outer positioning of the opening.

Smaller bottleneck mounted on the holding plate

The smaller bottleneck (diameter 42 mm) can be inserted to the inner position.

Standard clamp and lab stand

The container system can be mounted with help of a standard clamp and a lab stand.

4 Connect and transfer

In this chapter

Safety precautions

NOTICE

It is not recommended to use connectors other than ReadyMate connectors that are 9.5 mm or larger, which can be used in jumper designs. Cytiva cannot guarantee that they will not block the transfer of Cytodex Gamma microcarriers.

NOTICE

It is not recommended to use other connectors. Cytiva cannot guarantee that they will not block the transfer of Cytodex Gamma microcarriers.

NOTICE

Cytiva recommends the use of the larger transfer tubing for faster transfer into the top of large bioreactors.

Gas pressure

Apply pressure to clear the dip tube from any remaining material. Apply 0.3 bar (4.4 psi) to 0.4 bar (6.6 psi) of pressure to the vent filter with either dry air or N_2. To apply pressure, only use a pipetting (Peleus) ball equipped with a manometer.

After the pressure is applied to the Cytodex Gamma container system , wait approximately five seconds to allow pressure build-up inside the container system.

CAUTION

Make sure not to generate pressures above 0.5 bar, especially when using the pipetting (Peleus) ball without a manometer.

Special connections

If special connections are needed between the Cytodex Gamma container system and the bioreactor or mixer, consult ReadyToProcess™ configurator at *cytiva.com*.

Any new design needs to be evaluated for risk of clogging before implementation.

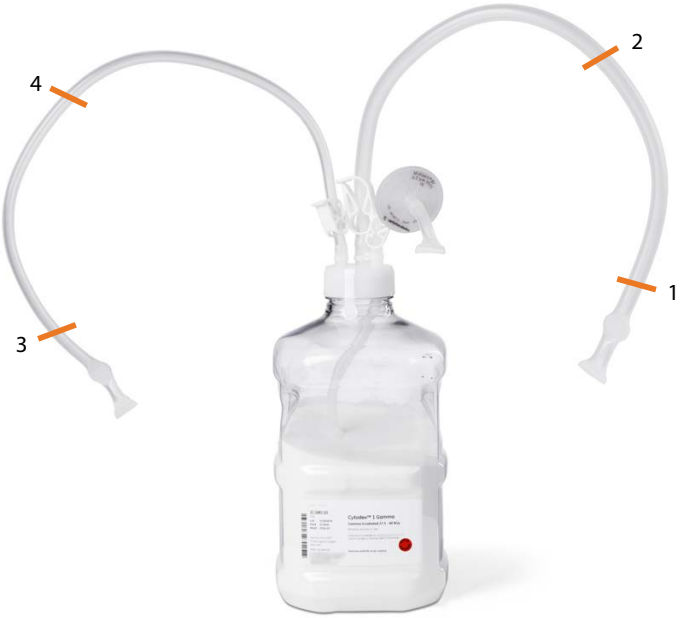
4.1 Connections

Connectivity options

Each container system can be connected aseptically at least four times. Any excess of Cytodex Gamma microcarriers can be pooled into another container.

The possible connections are listed below and the corresponding cutting points are shown in the figure:

- welding of the 9.5 mm ID C-Flex tubing. As the tubing is 75 cm in length it can be welded at least twice (1, 2)
- welding of the 6.4 mm ID C-Flex tubing. As the tubing is 75 cm in length it can be welded at least twice (3, 4)



NOTICE

Poor welding can block the transfer of Cytodex Gamma microcarriers.

Select connection

Select tubing suited to the volume of Cytodex Gamma microcarriers that will be transferred:

- for large volumes, 3 kg pack size, use the 9.5 mm ID C-Flex tubing.
- for other transfers, 30 to 300 g, any of the connectivity options can be used.

Note: *It is important to perform the connections according to these instructions in order to maintain sterility and aseptic transfer of Cytodex Gamma microcarriers.*

NOTICE

All connections should be as short as practically possible to minimize the risk of clogging during transfer. This is especially important for the Cytodex Gamma 3 kg pack size. Also with bioreactor setups, where there is a risk of moisture in the receiving connecting tubing, the tube needs to be as wide as possible. This to prevent clogging upon potential microcarrier swelling.

NOTICE

It is not recommended to use connectors other than ReadyMate connectors that are 9.5 mm or larger, which can be used in jumper designs. Cytiva cannot guarantee that they will not block the transfer of Cytodex Gamma microcarriers.

NOTICE

Make sure the transfer tubing is aligned horizontally to prevent clogging of the connections.

Container-to-container

An empty container system can be used to connect a new container system for container-to-container transfer. In this case, a remaining sterile transfer tubing on the container system can be used.

Symbols

The following colored symbols are used in these instructions to clarify the transfer process.

Item	Color	Description
	Green	Open pinch clamp

Item	Color	Description
	Red	Closed pinch clamp
	Blue	Cytodex Gamma microcarrier flow
	Orange	Gas flow

4.2 Connect and start transfer

Single container system

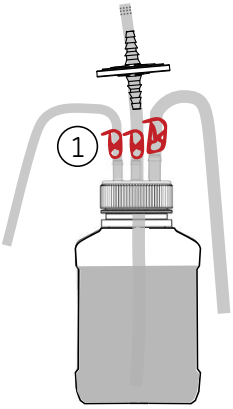
Follow the instructions below to connect a Cytodex Gamma container system to a bioreactor and to start the transfer.

Select a connection according the description in *Connectivity options, on page 35*.

Note: *It is advisable to have some culture media inside the bioreactor prior to microcarrier transfer. This helps prevent the risk of cake formation in pockets and dead ends, which can be difficult to mix into suspension.*

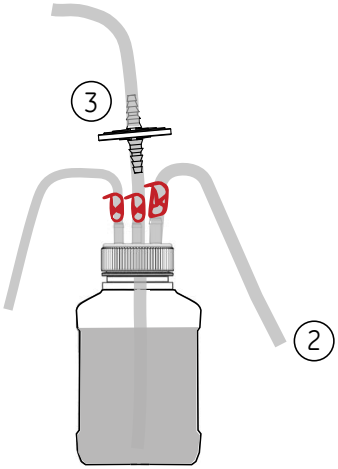
Note: *The instructions below describe a connection made by welding.*

Step	Action
1	Check if the tube to be welded is clear of any Cytodex Gamma. If there is any material in the tube then move it back to the container by flicking and shaking the tubing.
2	Check that all pinch clamps are closed and positioned close to the outlets on the ported cap (1).

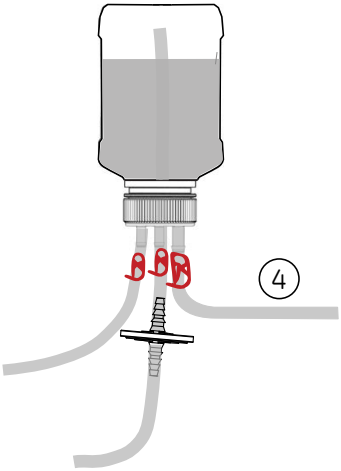


3	Connect the container system to the bioreactor by welding two complementary C-Flex tubes together.
4	Remove the protective tubing from the vent filter.

Step	Action
5	Connect the gas line to the vent filter (3).

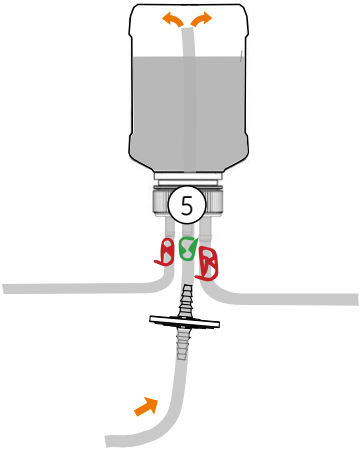


6	Turn the Cytodex Gamma container system upside down and place and immobilize the inverted container system in a holder.
7	Align the transfer tubing horizontally throughout the transfer line (4).



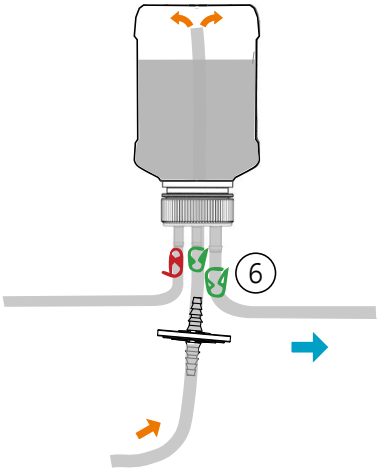
8	Make sure the dip tube is clear of the settled microcarrier bed. If not, shake the container, or place the container system upright and start over.

Step	Action
9	Open the pinch clamp on the vent line (5).



10	Apply gas via the vent filter, see *Gas pressure, on page 33*. Make sure the vent line and the dip tube are blown free from microcarriers.
11	Check that the bioreactor is not under pressure, and that the vent filter is open.
12	Wait roughly five seconds to allow for pressure build-up inside the container system.

Step	Action
13	Open the pinch clamp on the transfer line (6) to start the transfer of microcarriers.



14	Monitor the transfer visually and constantly check that the microcarriers running freely without clogging the line. If clogging occurs then gently squeeze the clogged part. To completely empty the container, take it out from the holder and turn it back and forth a couple of times

NOTICE

Monitor the transfer process visually to make sure no clogging occurs in the ported cap or in the transfer line. If severe clogging occurs, remove the pressure, place the bottle upright and squeeze the blocked material back into the container. Then start the procedure again.

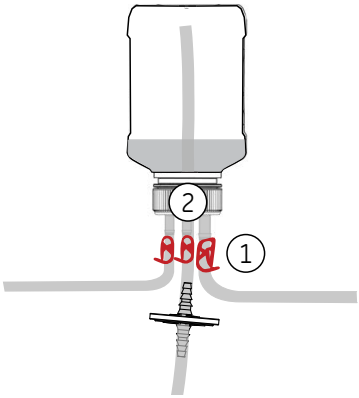
4.3 Stop transfer

Single container

Stop the transfer when the required amount of microcarrier is transferred to the bioreactor.

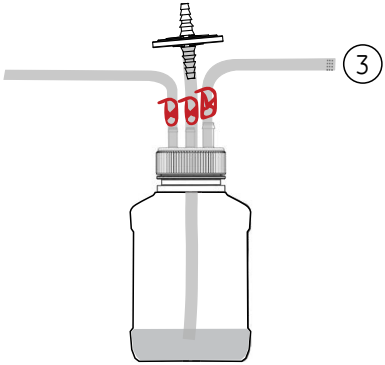
Note: *The instructions below use a welded connection.*

Step	Action
1	Close the pinch clamp on the transfer line (1) when the required volume is reached, or total volume has been added.
2	Stop the gas flow by closing the pinch clamp on the vent line (2).

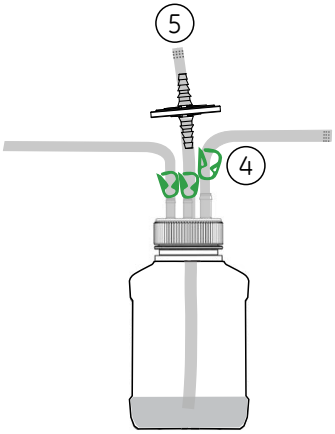


3	Disconnect the gas, or the pipetting (Peleus) ball from the vent filter. Remove the gas line.
4	Place the Cytodex Gamma container system upright. Make sure to empty the tubing as completely as possible from any remaining material as it will interfere with sealing.

Step	Action
5	Seal off the bioreactor and the transfer line (3) to maintain sterility.



6	Open all pinch clamps (4) and put back the protective tubing on the vent filter (5). Keep any remaining microcarrier in storage.



7	Store Cytodex Gamma container system dry for reuse.

4.4 Equilibration

Swell and equilibrate

Continue the procedure by adding cell culture medium to the bioreactor. Allow Cytodex Gamma microcarriers to swell and equilibrate for at least two hours during stirring before adding cells, depending on your application, i.e. cell type and cell culture media formulation.

5 Dosing

In this chapter

Dosing of Cytodex Gamma microcarriers

The required amount of Cytodex Gamma microcarriers can be transferred by using weight or volume methods.

The following methods are available:

- an empty container and weighing in the required amount
- a certain length or volume of the connected tube can be used to dose smaller volumes
- the bioreactor load cell if sensitive enough for the amount to be transferred
- the weight decrease registered from a container hanging from a balance

5.1 Empty container system

Setup

An empty container system can be used to weigh in a specific amount of Cytodex Gamma microcarriers, which can be used to feed a defined bioreactor volume.

Note: *Any sterile connections and reuse of container systems, is solely a customer responsibility, and something which Cytiva cannot be held responsible for.*

Mounting example

NOTICE

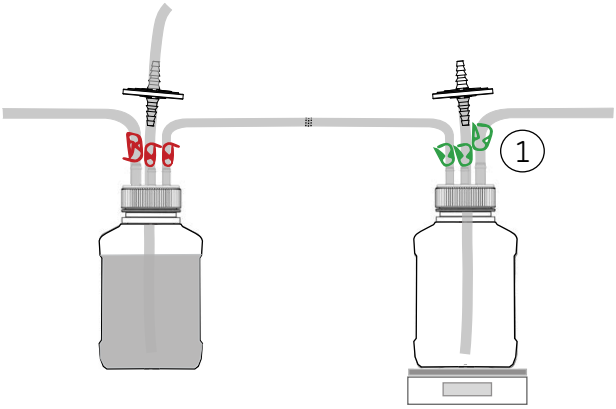
Make sure the transfer tubing is aligned horizontally to prevent clogging of the connections.

The figure below shows an example how an empty container can be mounted. Align the transfer tubing horizontally throughout the transfer line.

Weighing of Cytodex Gamma microcarriers

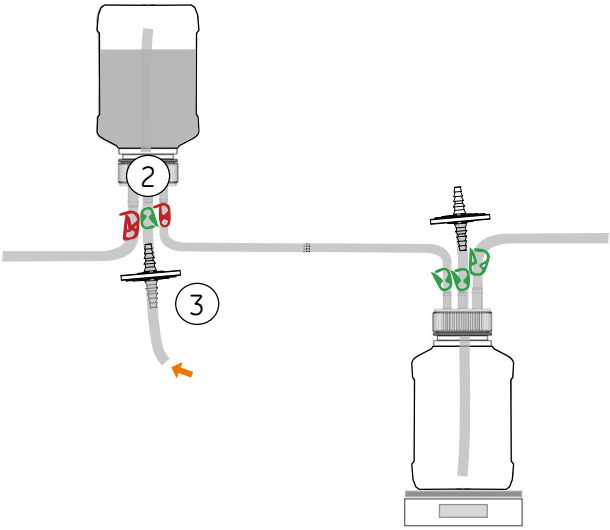
The instructions below describe an aseptic weighing of Cytodex Gamma microcarriers from a new container system with help of a container system already connected to a bioreactor.

Step	Action
1	Place the receiver container system on a balance. The pinch clamps on the receiver container must be open (1).



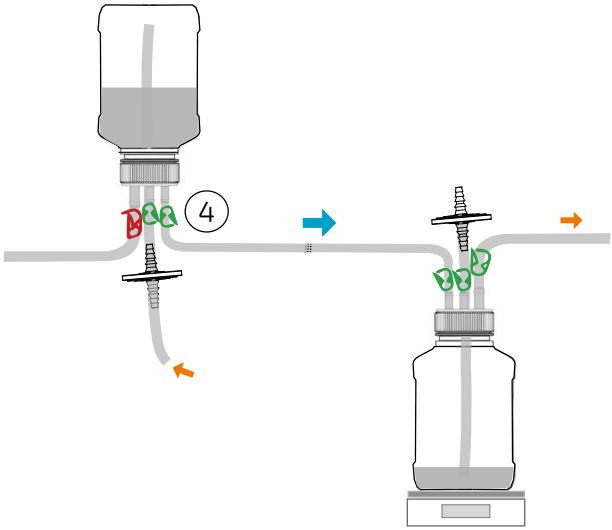
2	Make sure the transfer tubing does not affect the taring of the balance.
3	Turn the feed container upside down. Make sure the dip tubing arises above the Cytodex Gamma microcarrier level. If not, shake the bottle, alternatively place it upright and start over.
4	Place and stabilize the inverted feed container in a holder.
5	Check that the receiving container system is not set under pressure.
6	Open the pinch clamp on the vent line (2).

Step	Action
7	Apply gas via the vent filter (3), see *Gas pressure, on page 33*.



8	Wait approximately five seconds to allow for pressure build-up inside the feed container system. Make sure the vent line and the dip tube are blown free from Cytodex Gamma microcarriers.

Step	Action
9	To start the transfer, open the pinch clamp on the transfer line (4). The receiver vessel connected to this container system must allow free circulation of gas.

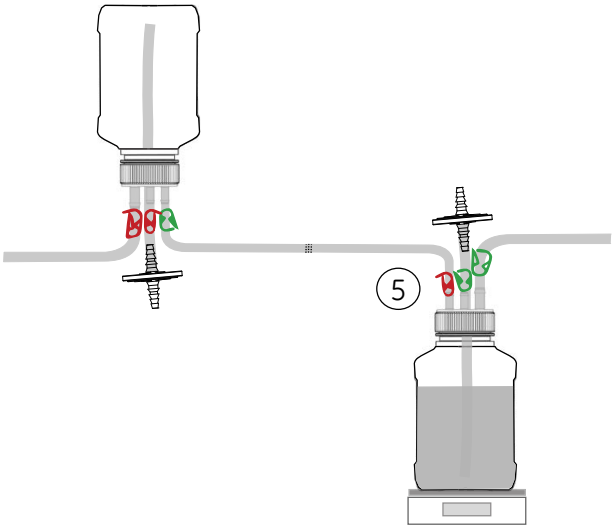


NOTICE

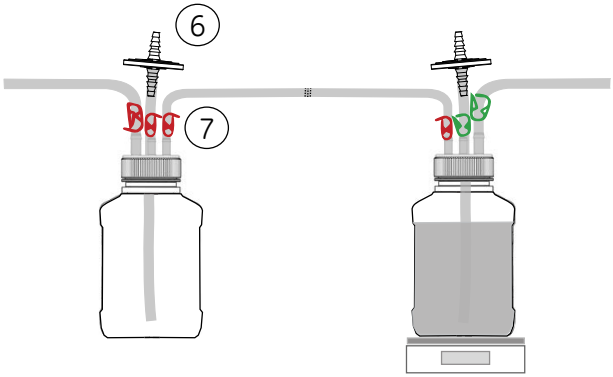
The dip tubing and the vent filter will be blocked by Cytodex Gamma microcarriers when standing in the upright position. When transferring Cytodex Gamma microcarriers between container systems, the second transfer line must be open and connected to another sterile vent filter, possibly via fermenter.

10	Visually monitor the transfer continuously to check that the Cytodex Gamma microcarriers are running freely and not clogging the transfer line. In case of clogging, gently squeeze the clogged part.
11	Slow down the transfer rate partially by using the pinch clamps on the transfer line when the transfer of Cytodex Gamma microcarriers is close to the required amount.

Step	Action
12	Close the pinch clamp on the connection between the feed container system and the receiver container system (5), when the required amount of Cytodex Gamma microcarriers is weighed.



13	Close the gas pressure. Remove the gas line from the vent filter, and close the clamp on the vent tubing.
14	Empty the connecting tube from the remaining Cytodex Gamma container system back into the feed container.
15	Disconnect the gas supply on the feed container system (6) and close the pinch clamp placed on the transfer line (7).



5.2 Length of tubing

Setup

Smaller amounts of Cytodex Gamma microcarriers can be determined with help of the transfer tubing.

The density of the Cytodex Gamma microcarriers is 0.77 g/ml, this means:

6.4 mm ID C-Flex tubing	Weight of 1 cm tube	Weight of 50 cm tube
Cytodex 1 Gamma	0.23 g	11.5 ± 0.5 g
Cytodex 3 Gamma	0.21 g	10.5 ± 0.5 g

9.5 mm ID C-Flex tubing	Weight of 1 cm tube	Weight of 50 cm tube
Cytodex 1 Gamma	0.56 g	28 ± 1 g
Cytodex 3 Gamma	0.48 g	24 ± 0.5 g

Transfer

Follow the instructions below to use the length of tubing to determine the amount of Cytodex Gamma microcarriers.

Step	Action
1	Determine the amount of Cytodex Gamma microcarriers to be added.
2	Decide which tubing to use.
3	Calculate the tubing length to be filled. Measure the length, and mark up the tubing with a lower and upper mark.
4	Connect the Cytodex Gamma container system to the bioreactor.

Step	Action
5	Close off the transfer tubing at the lower mark with a hemostatic forceps.

6	Fill the tubing between the marks.
7	Close off the tubing at the upper mark with a hemostatic forceps.

Step	Action
8	Open the tubing at the lower mark and transfer the Cytodex Gamma microcarriers into the bioreactor. 

5.3 Bioreactor load cell

Setup

A bioreactor load cell can be used for dosing of Cytodex Gamma microcarriers if the sensitivity is good enough for the amounts of material to be transferred.

Transfer

Follow the instructions below to use a bioreactor load cell to measure the amount of Cytodex Gamma microcarriers.

Step	Action
1	Connect the feed Cytodex Gamma container system and the bioreactor.

2	Fix the bioreactor inlet tubing so it does not affect the taring of the bioreactor.
3	Tare the bioreactor.
4	Check that the vent filter inside the bioreactor is open and the bioreactor is not pressurized.
5	Start the transfer by applying gas via the vent filter on the Cytodex Gamma container system , see *Gas pressure, on page 33*.
6	Align the transfer tubing horizontally throughout the transfer line.
7	Slow the transfer rate, by partially closing off the transfer line close to the bioreactor, when the load cell indicates that the final amount is close.
8	Stop the transfer when the required volume is reached.

5.4 Hanging from a balance

Setup

The decrease in weight can be registered by hanging a container from a balance.

Note: *Precision balances can be equipped with a hook to weigh hanging materials.*

Transfer

Step	Action
1	Place the Cytodex Gamma container system in a plastic bag with the tubing protruding through holes in the bag.
2	Mount the suspended digital balance on a holder.
3	Connect the Cytodex Gamma container system to the bioreactor.
4	Close all pinch clamps.
5	Apply pressure via the vent filter, see *Gas pressure, on page 33*.
6	Hang the container from the balance and tare the balance.
7	Start the transfer by opening the pinch clamps on the vent line and the transfer line.
8	Check the decrease in weight which corresponds to the amount of material transferred to the bioreactor.
9	Align the transfer tubing horizontally throughout the transfer line.
10	Slow the transfer rate, by partially closing the inlet tubing close to the container, when the amount of Cytodex Gamma microcarriers transferred is near to the required weight.
11	Stop the transfer when the required weight is reached.

6 After transfer

In this chapter

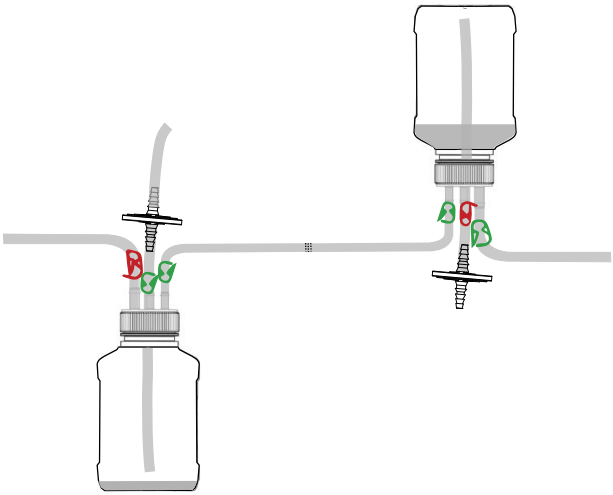
6.1 Pooling

Pooling of Cytodex Gamma microcarriers

Remaining Cytodex Gamma microcarriers from a transfer, can be pooled from a container to the feed container. In this case, the vent filter on the bioreactor and the transfer tubing must be open to let the gas escape.

If necessary, more container systems can be linked in a series to a pooling container.

Note: *Any sterile connections and reuse of container systems, is the sole responsibility of the customer, and something which Cytiva cannot be held responsible for.*



6.2 Disconnect

Seal the bioreactor tubing

The transfer line must be disconnected when the transfer of Cytodex Gamma microcarriers is finished.

Before disconnecting the container system, carefully empty the tubing of any remaining Cytodex Gamma and close the bioreactor tubing by sealing. Then follow the welding instructions below.

If welding is used

If...	Then...
no Cytodex Gamma microcarriers remain and you do not wish to use the Cytodex Gamma container system for further use immediately.	• disconnect and discard the Cytodex Gamma container system , or • seal closed to save the container system for pooling purposes later on.
Cytodex Gamma microcarriers remain in the container system.	• seal all tubing on the Cytodex Gamma container system . • disconnect. • fit the protective tubing on the vent filter. • store as is, or connect and pool remaining material into a pooling bottle.

Note: *A Vante™ TPE Sealer System can be used to seal off the tubes, providing that the inside of the tubing is clear of Cytodex Gamma.*

6.3 Storage

Instruction

The user is responsible for maintaining proper Cytodex Gamma container system function by following the storage instructions.

NOTICE

Pinch clamps should not be closed during storage as the resulting deformation of the C-flex tubing can block transfer when opened after long-term storage.

The following rules apply for storage of the Cytodex Gamma container system :

- The container system must be stored upright and with welded seals or clamps to maintain sterility.
- The protective tubing must be attached to the vent filter after use.
- All pinch clamps should be opened to prevent deformation of tubing during storage.
- The container system must be stored at room temperature in a dry environment. This prevents a potential increase in moisture content, that can cause clogging and problems with material transfer at a later stage.
- The Cytodex Gamma container system should not be used if the expiry date has passed.
- Protect against sunlight.

6.4 Disposal

Disposal of Cytodex Gamma microcarriers and the packaging

Always follow the local regulations for handling and disposal. Cytodex Gamma container systems are designed to be disposed intact as combustible material.

7 Troubleshooting

Cytodex Gamma container system troubleshooting

Problem	Possible cause	Corrective action
Cytodex Gamma microcarriers are not transferred to the bioreactor.	The tubing is clogged.	• Shut off the gas stream. Place the container system in an upright position, and let the Cytodex Gamma microcarriers return to the container from the tubing. Check the welding i.e. re-weld. Switch on the gas again and resume the Cytodex Gamma microcarrier transfer into the bioreactor. • Align the transfer tube in a horizontal position. • Squeeze the tubing to dissolve the clogging. • Use as short and wide connections as possible to speed up the transfer and to minimize any clogging tendencies.
	The pressure is too low or too high pressure.	Check the pressure.
	Poorly welded tubing.	Reweld.
Slow initial attachment.	Ethanol concentration is too high inside the microcarrier beads.	Increase equilibration time prior to cell inoculation.
Problems with cell growth/ virus titer.	Polysaccharides are present on the microcarrier beads.	Swell and wash the microcarrier beads in sterile PBS prior to equilibration and use.

Cytodex Gamma container system troubleshooting

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Slow initial attachment.	Ethanol concentration is too high inside the microcarrier beads.	Increase equilibration time prior to cell inoculation.
Problems with cell growth/ virus titer.	Polysaccharides are present on the microcarrier beads.	Swell and wash the microcarrier beads in sterile PBS prior to equilibration and use.

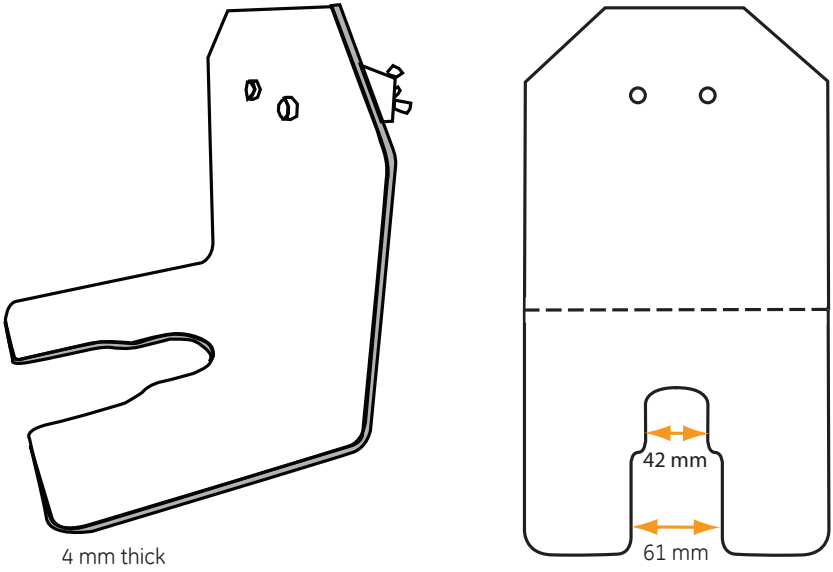
8 Reference information

In this chapter

8.1 Holding plate design

Holding plate

The figure below shows how a holding plate can be designed with the most important dimensions.



8.2 Ordering information

Cytodex Gamma container system products

Product	Pack size	Container volume	Code No
Cytodex 1 Gamma	30 g	125 ml	17548701
Cytodex 1 Gamma	300 g	500 ml	17548702
Cytodex 1 Gamma	3 kg	5 L	17548703
Cytodex 3 Gamma	30 g	125 ml	17548801
Cytodex 3 Gamma	300 g	500 ml	17548802
Cytodex 3 Gamma	3 kg	5 L	17548803

Other

For jumpers and connections with ReadyMate connectors that are 9.5 mm and larger, see the ReadyToProcess configurator: *cytiva.com*.

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