Instructions for Use

Small-Scale Microcarrier Culture

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SARTORIUS

Contents

1 Physical Characteristics of SoloHill® Microcarriers

Microcarrier Type, Core Material and Surface Chemistry	Part Number Prefix	Relative Density Range	Size (microns)	Surface Area (cm^2/g)	Surface Charge	Protein Coated	Number of MC per Gram
Plastic Cross-linked polystyrene	P-221	1.022 – 1.030	125 – 212	360 cm^2	No	No	4.6×10^5
	P-215	1.022 – 1.030	90 – 150	480 cm^2	No	No	1.0×10^6
	P-421	1.034 – 1.046	125 – 212	360 cm^2	No	No	4.6×10^5
Plastic Plus Cross-linked polystyrene, cationic-charged	PP-221	1.022 – 1.030	125 – 212	360 cm^2	Yes	No	4.6×10^5
Star-Plus Cross-linked modified polystyrene, cationic-charged	SP-221	1.020 – 1.035	125 – 212	360 cm^2	Yes	No	4.6×10^5
Hillex® II Modified polystyrene, cationic-charged	H-170	1.080 – 1.150	150 – 210	515 cm^2	Yes	No	5.5×10^5
Collagen Cross-linked polystyrene coated with Type 1 porcine collagen (gelatin)	C-215	1.022 – 1.030	90 – 150	480 cm^2	No	Yes	1.0×10^6
	C-221	1.022 – 1.030	125 – 212	360 cm^2	No	Yes	4.6×10^5
	C-421	1.034 – 1.046	125 – 212	360 cm^2	No	Yes	4.6×10^5
FACT III Cross-linked polystyrene coated with Type 1 porcine collagen (gelatin), cationic-charged	F-221	1.022 – 1.030	125 – 212	360 cm^2	Yes	Yes	4.6×10^5

Table 1: List of SoloHill® Microcarrier types and their properties

Microcarrier types may be purchased with a combination of physical and chemical properties listed in the table above. They are offered in multiple standard-sized formats ranging from 10 g to 1000 g quantities. SoloHill® Microcarrier types do not require any hydration or rinse steps prior to their use in the cell culture processes.

Microcarriers are available in two different formats: non-sterile and sterile, ready- to-use (gamma-irradiated). SoloHill® Microcarriers provide an excellent substrate for large-scale cell expansion and production of vaccines, biologics and cell and gene therapy applications. Contact Sartorius for additional information at microcarriers@sartorius.com.

2 Equipment, Reagent, and Material List

2.1 Equipment

- Biosafety cabinet (BSC)
- Magnetic stir-plate (Bellco Glass 7785-D9005, 7785-D2000)
- Spinner flasks
  - Glass re-usable spinner flasks
    - 125 mL (Corning 4500-125)
    - 250 mL (Corning 4500-250)
  - Disposable spinner flasks
    - 125 mL (Corning 3152)
    - 500 mL (Corning 3153)
- Hemocytometer or cell | nuclei counting device
- Vortex mixer
- Humidified Incubator
- Water bath or Heat block (dry bath)
- Pipettor
- Analytical balance

2.2 Reagent

- Cell culture medium
- Cell disassociation enzyme
- Dulbecco's Phosphate Buffered Saline (DPBS), Ca++- and Mg++-free
- Nuclei release solution (i.e., DI water containing 0.1M Citric acid, 0.05% Triton X-100, 0.1% w/v Crystal violet)
- Sigmacote® (Millipore Sigma SL-2)
- Alcojet (Alconox Inc. 1404)

2.3 Materials

- 24-well Tissue culture plates (VWR 62406-183)
- Sterile Conical tubes, 15 and 50 mL
- Sterile spatula
- T-25 Tissue culture flasks
- Serological pipettes (1, 5, 10, 25 and 50 mL)
- 70 µm disposable cell strainers (Corning 352350)
- Stainless steel sieves (Bellco Glass 1985-11300 and 1985-00200)
- 250 mL polypropylene beaker (VWR 13915-103)
- Autoclavable container (60 – 100 mL) (VWR 16120-737, 16120-741)

3 Procedure

3.1 Siliconizing Cell Culture Glassware

We recommend treating all glass surfaces used for cell culture with Sigmacote® to prevent cell attachment on the glassware. Follow the manufacturer's instructions, summarized below.

Start with Clean Glassware

1. Apply the Sigmacote® product (approximately 20 mL for 250 mL container) and swirl to thoroughly coat the surface of all glassware to be exposed to cells and microcarriers.
2. Drain the excess amount of Sigmacote® and allow the glassware to air-dry for 18 – 24 hours.

Tip

It is optional to bake the glassware in the oven at 95°C for ½ hour after the air-drying step. Bake the glass portion of the re-usable spinner flask **only**.

3. Wash coated glassware with a mild detergent routinely used for labware cleaning (1% Alcojet) and perform a final thorough rinsing with the de-ionized (DI) water.
4. Clean and maintain cell culture glassware with tissue culture approved solutions only.
5. Routinely monitor glassware to assure the maintenance of the coating.
   – Aqueous solution should form beads that are repelled from coated surfaces.

3.2 Autoclaving Standard Microcarriers

The following process is written as a guideline for 200 mL 5 cm^2/mL density Plastic (125 – 212 micron) microcarrier culture. The optimal microcarrier density and sterilization condition may vary from process to process. Process optimization and validation of sterility should be performed by the end user.

The equation to calculate the microcarrier amount for desired culture volume.

Culture volume (mL)		Microcarrier density (cm^2/mL)		Microcarrier surface-area per gram (cm^2/g)		Microcarrier amount (g)
200	×	5	÷	360	=	2.78

1. Weigh out 2.78 g of microcarriers and transfer them to an autoclavable container.
2. Add 20 mL of DI water to the container and gently swirl to wet microcarriers.
3. Autoclave microcarriers for 30 minutes at 121°C.
4. Transfer autoclaved container containing microcarriers in the BSC and allow microcarriers to settle to the bottom of the container.
5. Using good aseptic technique, carefully pipette out the residual DI water from the container without removing a significant amount of microcarriers.
6. The microcarriers are ready to be used for cell culture.

3.3 Handling of Gamma-Irradiated Microcarriers

1. Tare a sterile 50 mL conical tube using an analytical balance.
2. Place the gamma-irradiated microcarriers, sterile spatula and tared 50 mL sterile conical tube in the BSC.
3. Transfer the desired mass of gamma-irradiated microcarriers from the stock container to the 50 mL conical tube using a sterile spatula.

Tip

For 2.78 grams of microcarriers, add sterile microcarriers up to ~2.5 mL mark in the 50 mL conical tube.

4. Close the conical tube containing the microcarriers and weigh to obtain the microcarrier mass transferred to the conical tube. Adjust mass as necessary by aseptically adding or removing the required mass to achieve the desired amount.

3.4 Microcarrier Acclimation in Cell Culture Medium

The following guideline assumes that the chosen cell growth medium supports efficient cell attachment to microcarriers under dynamic conditions. If a particular cell type displays challenges with attachment under dynamic conditions, refer to section 8 for strategies that may improve cell attachment on the microcarriers.

1. Obtain a re-usable sterile or single-use disposable spinner flask and place it in the BSC.
2. Position the spinner flask on a magnetic stir plate and set the appropriate agitation speed for the microcarrier type. Refer to Table 2 below.

Microcarrier Type	Agitation speed revolution per minute (rpm)
Plastic, Plastic Plus, Star-Plus, Collagen, Fact III	35 – 45 rpm
Hillex® II	55 – 60 rpm

Table 2: Recommended stir speeds for SoloHill® Microcarrier types.

   - The above agitation speeds represent a good starting point for individual microcarrier culture. The optimal agitation speed may vary based on the physical geometry of the vessel used for the microcarrier culture.
   - A general rule of thumb is to mix at the lowest possible speed that allows for a relatively uniform suspension of microcarriers throughout the culture vessel. If microcarriers begin to settle at the bottom of the vessel, gradually increase the agitation rate in small increments (~2 – 5 rpm) to maintain uniform mixing as much as possible. It is not necessary to achieve a completely gradient-free microcarrier distribution throughout the suspension.

3. Add 20 mL of cell culture medium to the sterile container of microcarriers prepared above (see chapter 3.2, step 6 or chapter 3.3, step 4).
   - If cell attachment is being performed at a low protein | serum concentration, add basal medium without serum | protein-supplemented at this step. If cell attachment is being performed at full protein | serum concentration levels, add growth medium supplemented with the desired concentration of serum | protein at this step.
4. Mix the microcarrier slurry using a sterile serological pipette and transfer it into the spinner flask.
5. Repeat step 3 (see chapter 3.4, step 3) with a second 20 mL of cell culture medium, transferring any remaining microcarriers into the spinner.
6. Add another 100 mL of cell culture medium to the spinner flask. This will bring the medium volume in the spinner to 140 mL.
7. Transfer the spinner flask to a humidified incubator and place it on the magnetic stir-plate. Acclimate microcarriers and medium for 30 – 120 minutes at the optimal agitation rate for the respective microcarrier type.

3.5 Cell Inoculum for Microcarrier Culture

To obtain a uniform distribution of cells across the microcarrier population, it is essential to generate a robust, single-cell suspension that is free of aggregates and clumps.

1. Aspirate cell culture medium from the tissue culture flask.
2. Rinse the culture flask twice with Ca++- and Mg++- free DPBS at 0.2 mL per cm^2 volume to surface area (SA) ratio.

Tip

For more strongly-adherent cell types, such as MDCK, it is recommended to rinse the cell layer with Ca++- and Mg++ -free DPBS containing 0.5 mM to 1 mM EDTA prior to enzyme addition.

3. Add cell dissociation solution to the culture flask at a volume to SA ratio of 0.04 to 0.06 mL per cm^2 and distribute evenly. Incubate the flask either at room temperature (RT) or 37°C.

Tip

To optimize viability when generating a single-cell suspension, consider:
- Titration of the dissociation enzyme.
- Evaluate dissociation using the various enzyme concentrations at both room temperature and 37°C.
- Choose the dissociation condition that results in the highest cell viability, while maintaining effective dissociation of the cells.

4. Monitor the cell dissociation microscopically, noting the time required for cells to become rounded and | or detached from the flask surface.
5. Gently tap the culture flask on the sides and confirm the cell dissociation process microscopically.
6. Add quench reagent to the culture flask at 0.08 to 0.12 mL per cm^2 volume to SA ratio and pipette the cell suspension to generate a single cell suspension throughout cell inoculum.
   - Examples of quench reagents include fresh medium, spent medium or DPBS with supplement (FBS or protein supplement).
7. Transfer the cell suspension to a sterile conical tube or bottle using a serological pipet.
8. Rinse the cell culture flask with a second volume of quench reagent at 0.08 to 0.12 mL per cm^2 volume to SA ratio and transfer remaining cells to the conical tube containing the cell suspension.
9. Thoroughly mix the cell suspension using a serological pipet and obtain a small, representative aliquot (300 µL) of cells to perform a cell count.

3.6 Cell Seeding and Expansion

1. Calculate the volume of the cell suspension required to seed individual spinner flask(s) at the desired seeding density. Typically, this is equivalent to the seeding density used to expand cells in planar culture.

Culture volume (mL)	×	Microcarrier density (cm^2/mL)	×	Cell seeding density (cells/cm^2)	÷	Concentration of viable cell suspension	=	Cell suspension volume (mL)

   - It is not recommended to seed MC cultures at seeding densities lower than 3×10^3 cells per cm^2. This seeding density equates to roughly 2 – 3 cells per microcarrier and ensures enough cells are seeded to promote an even distribution of cells across the MC population. Seeding below this threshold may result in a significant portion of MC remaining unoccupied throughout the culture.
2. Gently mix the cell suspension using a serological pipette and aliquot the required volume into the individual sterile conical tube(s).

3. Centrifuge the cell suspension at the desired speed & temperature settings, remove harvest medium and resuspend the cell pellet in 10 mL of cell culture medium.
   - Gently resuspend cell pellet without forming an excessive amount of bubbles to minimize shear stress on cells.
   - If cell attachment is being performed at a low protein | serum concentration, resuspend the cells into a medium that contains the desired concentration of protein | serum. For example, when seeding a 200 mL microcarrier culture at 0.05% serum | protein, resuspend the cell pellet in a 10 mL basal medium containing 100 µl of serum | protein supplement.
   - If cell attachment is being performed using complete medium (full serum | protein concentration), resuspend the cells into growth medium supplemented with the desired concentration of serum | protein.
4. Remove the spinner flask from the incubator and place it in the BSC on the stir plate set at the desired agitation rate (see Table 2).
5. Allow the microcarrier suspension to mix well in the spinner flask and transfer the cell inoculum into each individual spinner flask using a sterile pipet.
6. Allow the microcarrier and cell suspension to mix briefly and collect a 0.5 mL representative sample using a 1 mL serological pipet.
   - To collect a representative sample, insert the pipette into the middle of the spinner before withdrawing the sample, being careful to not impede the spinner impellor.
7. Transfer the microcarrier culture sample to one well of a 24 well plate for microscopic evaluation. This plate may also serve as a static microcarrier culture control.
8. Transfer the spinner flask to the 37°C incubator on the stir plate and mix the culture at optimal agitation speed (see Table 2).
9. Seed multiple tissue culture flasks (T-25 flask) as planar culture controls.
10. Collect samples periodically within the first 4 hours of cells seeding to evaluate and document cell attachment across the microcarrier population.

Tip

Optimizing cell attachment conditions on microcarriers reduces the time it takes for the cells to attach and spread on the MC surface. The longer the cells remain in suspension, the more likely they will aggregate with each other prior to attaching to the MC. This can lead to uneven cell distribution and confluency differences among microcarriers.

A. Strategies have been identified that can improve or speed up initial cell attachment on microcarriers and are listed below:

B. Reduction in total protein or serum concentration during the cell attachment phase of the microcarrier culture.
   - Once the majority of cells have attached to the microcarriers, serum or protein supplementation can be added back to the desired concentration for the remainder of the culture duration. The optimal concentration of reduced serum | protein concentration for cell attachment is a cell dependent phenomenon and needs to be determined empirically.
   - Reduced serum | protein concentration of 0.1 to 0.05% have been successfully used with multiple cell types to achieve efficient cell attachment within 2 – 4 hours of cell inoculation.

C. In cases where a reduction in serum | protein concentration cannot be employed for cell attachment, the following tips may be helpful:
   - Perform a reduced-volume attachment. This effectively increases the MC concentration and thus increases the number of cell and MC interactions during the attachment phase. Once acceptable cell attachment has been achieved, bring microcarrier concentration to its working range by adding the appropriate volume of medium for the remainder of culture.
   - Remove cell detachment enzyme and its inhibitor from the harvested cell suspension via centrifugation or another filtration step before seeding on the microcarrier culture.
   - Employ an interval mixing mode for agitation during the cell attachment phase. Once the majority of cells are attached to the microcarriers, switch mixing to continuous stirring mode for the remainder of the culture.

11. Once cells have attached and spread on microcarriers,
    - Add any supplements necessary to bring their concentrations to normal levels if a reduced-supplement | protein attachment was performed.
    - Bring the total volume of the culture to a final volume of 200 mL.
12. Daily sampling is recommended to monitor cell growth throughout the culture.
13. To perform a medium exchange, transfer the flask from the incubator to the BSC and allow microcarriers to settle to the bottom of the flask.
    - Optimal medium exchange and supplementation requirements vary from for each cell and medium combination and strategies should be determined empirically.
14. Aseptically remove the spent culture medium from the vessel without disturbing the cell-laden microcarrier pack.
15. Gently add the same volume of fresh cell growth medium to the flask without generating excessive foam using a serological pipette.
    - Add fresh growth medium to the side of the flask using a steady stream.

3.7 Sampling and Quantification of Viable Cell Density

The following process is written as a guideline for 200 mL cultures containing Plastic microcarriers (125 – 212 micron) at a density of 5 cm^2/mL. The harvest process should be optimized for each cell type.

1. Remove spinner flask(s) from the 37°C incubator and place it in a BSC on a stir plate which is set at the desired agitation rate. (see Table 2)
2. Allow microcarrier suspension to mix well in the spinner flask before withdrawing a representative sample.
    - Insert the pipette to the middle of the spinner flask to retrieve the sample, being careful not to impede the impellor.
3. Collect a 5 mL sample from each spinner flask and transfer it into 15 mL sample tubes.
4. Transfer spinner flasks back into the 37°C incubator, place on a stir plate and mix the culture at optimal agitation speed.
5. Allow cell-laden microcarriers in the 15 mL sample tube to settle and aspirate supernatant.
    - Retain spent medium if using to quench harvest enzyme.
6. Add 5 mL of DPBS (Ca++- and Mg++- free) or optimal wash buffer to the sample, thoroughly mix and allow microcarriers to settle.
7. Aspirate the wash buffer and repeat a DPBS rinse process.
8. Add 1 to 1.5 mL of harvest enzyme, pre-warmed at 37°C, to the sample tube, thoroughly mix and incubate at room temperature.
9. Mix microcarrier sample every 2 to 3 minutes by inverting the sample tube.
10. Observe the microcarrier sample after 5 to 10 minutes using an inverted microscope to evaluate if cells are rounded or are on the verge of dislodging from the microcarriers.
    - Samples can be observed directly in the 15 mL tube or by transferring a small amount of sample to a 24-well plate. Time for cell dissociation will vary depending on the cell type and the confluent density of the culture and must be carefully observed and optimized.

11. Pipette microcarrier sample gently up and down to dislodge cells from microcarriers and create a single-cell suspension.
    - Thoroughly mix and incubate the sample for an additional 3 to 5 minutes if cells have not disassociated from the microcarriers. Repeat pipetting until a satisfactory number of cells are detached from the microcarriers.

      NOTE Be sure to limit enzyme incubation time to avoid a negative impact on the cell viability.

12. Transfer cells and microcarrier slurry to a 70 µm cell strainer fitted onto a 50 mL conical tube and allow cells to pass through the strainer.
    - Weigh and record tare weight of strainer prior to usage for use in calculations performed in chapter 3.7, step 17.
13. Transfer 2 – 3 mL of quench medium into original the 15 mL sample tube to rinse and transfer contents to the strainer allowing the solution to flow into the 50 mL tube containing harvested cells.
14. Measure the total cell suspension volume and perform cell count on the collected cells.
    - The suspension can be diluted or concentrated to ensure cell concentration is within the recommended range of the cell counting device.
15. Calculate a viable number of cells harvested from the microcarrier sample using this formula.

Viable cell concentration, cells per mL	×	Cell suspension volume, mL	=	Total viable cells

16. Place the cell strainer containing microcarriers into a glass vessel of a known weight and either air dry the microcarriers overnight at room temperature or transfer to a 70°C oven to assure samples are completely dry.
17. When the samples are dried, weigh each on an analytical balance. Calculate the microcarrier mass by subtracting the combined weight of the cell strainer and glass vessel from the total weight of the sample.
18. Calculate the total surface area of the microcarrier sample using this formula.

Microcarrier weight, grams	×	Microcarrier surface-area per gram, cm^2/gram (Table 1)	=	Total surface-area

19. Estimate the viable harvest yield (cells per cm^2) of the microcarrier culture using this formula.

Total viable cells	÷	Total surface-area	=	Viable harvest yield, cells per cm^2

20. Cell harvest yield can be converted to cells per mL using this formula.

Viable harvest yield, cells per cm^2	×	Theoretical surface-area per unit volume, cm^2 per mL	=	Viable harvest yield, cells per mL

The theoretical surface area per unit volume is the microcarrier density (cm^2/mL) used to initiate the microcarrier culture. For example, the theoretical surface area per unit volume for a 200 mL culture run at 5 cm^2/mL is 5 cm^2.

3.8 Sampling and Quantification of Cell Density Using Nuclei Release Method

The following process is written as a guideline for 200 mL cultures containing Plastic microcarriers (125-212 micron) at a density of 5 cm^2/mL. The nuclei release process should be optimized for each cell type. For example, a nuclei release solution for Vero cells containing 0.1M Citric acid, 0.5% v/v Triton-X100 and 0.1% w/v Crystal violet in the DI water may not be appropriate for fibroblast-like cells.

1. Remove spinner flasks from the 37°C incubator and place them in a BSC on a stir plate set at the desired agitation rate (see Table 2).
2. Allow microcarrier suspension to mix well before withdrawing a representative sample.
   — Insert the pipette to the middle of the spinner flask to collect the sample, being careful not to impede the impellor.
3. Collect 5 mL samples from each spinner flask and transfer into 15 mL sample tubes.
4. Transfer spinner flasks back into the 37°C incubator.
5. Allow cell-laden microcarriers in the 15 mL sample tubes to settle and aspirate supernatant.
6. Resuspend the cell-laden microcarriers into a known volume of nuclei release solution.
   — The volume of nuclei release solution used can be equivalent to, lower than or greater than, the original supernatant volume removed from the microcarrier sample.
7. Vortex the microcarrier samples on high speed for one minute to facilitate nuclei release from cells attached to the microcarriers.
   — Incubation at 37°C for 1 hour or longer may be required to facilitate efficient nuclei release from cells attached on the microcarriers.
8. Transfer nuclei and microcarrier slurries to 70 µm cell strainers fitted onto a 50 mL conical tubes and allow the nuclei to pass through the strainers.
   — Weigh and record the tare weight of strainer prior to usage for use in calculations performed in chapter 3.8, step 12.
9. Measure the total volume of the suspension and perform a nuclei count.
   — The suspension can be diluted or concentrated to ensure nuclei concentration is within the recommended range of the counting device.
10. Calculate total nuclei released from the microcarrier samples using this formula.

Nuclei concentration, nuclei per mL	×	Total sample volume, mL	=	Total nuclei

11. Place the cell strainers containing microcarriers into a glass vessel of a known weight and either air dry the microcarriers overnight at room temperature or transfer to a 70°C oven to assure samples are completely dry.
12. When the samples are dried, weigh each on an analytical balance. Calculate the microcarrier mass by subtracting the combined weight of the cell strainer and glass vessel from the total weight of the dried sample.
13. Calculate the total surface area of the microcarrier sample using this formula.

Microcarrier weight, grams	×	Microcarrier surface-area per gram, cm^2/gram	=	Total viable cells

14. Estimate the cell density (nuclei per cm^2) of the microcarrier culture using this formula.

Total nuclei	÷	Total surface-area	=	Cell density, nuclei per cm^2

   — The theoretical surface area per unit volume is the microcarrier concentration (cm^2/mL) used to initiate the microcarrier culture. For example, the theoretical surface area per unit volume for a 200 mL culture run at 5cm^2/mL is 5cm^2.

3.9 Spinner Culture Harvest

The following process is written as a guideline for a 200 mL 5 cm^2/mL Plastic (125-212 micron) microcarrier culture. The harvest process typically varies from cell-to-cell and will need to be optimized.

1. Remove spinner flasks from the 37°C incubator and transfer to a BSC. Allow cell-laden microcarriers to settle and remove the spent medium.
   - Retain spent medium if using to quench the harvest enzyme.
2. Carefully add 200 mL of DPBS (Ca++- and Mg++-free) or optimal wash buffer to the spinners, mix and allow microcarriers to settle.
   - Add wash buffer to the side of the spinner flask using a steady stream.
3. Remove the wash buffer supernatant and repeat the buffer rinse.
4. Add 40 to 60 mL of harvest enzyme (pre-warmed at 37°C), thoroughly mix the microcarrier-enzyme suspension and incubate at room temperature.
   - Tightly adherent cell types, such as MDCK or highly confluent microcarrier cultures may benefit from incubation at 37°C to dislodge healthy cells from microcarriers in an efficient manner.
5. Thoroughly mix the microcarrier suspension every 2 to 3 minutes by placing the flask on a stir-plate set at the same agitation speed used during the cell growth phase.
   - If the total volume of the suspension is below the level of the impeller, mixing by gently swirling the spinner is sufficient to thoroughly mix the suspension.
6. Transfer a small amount of microcarrier sample from the spinner to a 24-well plate.
7. Observe the microcarrier sample using an inverted microscope and evaluate if the cells are becoming rounded or are on the verge of dislodging off the microcarriers.
   - Time for cell dissociation will vary depending on the cell type and the confluent density.
8. Pipette microcarrier culture gently up and down to dislodge the cells into a single-cell suspension.
   - Thoroughly mix and incubate the sample for an additional 3 to 5 minutes if cells have not disassociated from the microcarriers. Repeat pipetting of the microcarrier suspension until a satisfactory number of cells are detached from the microcarriers.
   - Be sure to limit enzyme incubation time to avoid a negative impact on cell viability.
9. Add 40 to 60 mL of quench reagent to the spinner flask to inhibit the harvest enzyme activity.
10. Mix the microcarrier suspension well and allow microcarriers to settle.
11. Transfer supernatant (containing cells) to a stainless-steel sieve (equipped with 70 µm screen) of known weight that is fitted onto a sterile 250 mL beaker, allowing cells to pass through the strainer.
12. Add another 40 to 60 mL of quench reagent to the spinner and transfer the entire content (cells and microcarriers) to stainless-steel sieve allowing the solution to flow into 250 mL beaker containing harvested cells.
    - An additional 10 mL of quench reagent can be added to wash remaining cells from the spinner flask to maximize cell recovery (Optional)
13. Measure the total cell suspension volume and perform a cell count on the collected cells.
    - The suspension should be diluted or concentrated to ensure cell concentration is within the recommended range of the cell counting device.
14. Calculate the viable number of cells harvested from the spinner flask using this formula.

Viable cell concentration, cells per mL ×	Cell suspension volume, mL	=	Total viable cells

15. Place the cell strainers containing microcarriers into a glass vessel of a known weight and either air dry the microcarriers overnight at room temperature or transfer to a 70°C oven to assure samples are completely dry.
16. When the samples are dried, weigh each on an analytical balance. Calculate the microcarrier mass by subtracting the combined weight of the cell strainer and glass vessel from the total weight of the dried sample.

17. Calculate the total surface area of the microcarrier using this formula.

Microcarrier weight, grams	×	Microcarrier surface-area per gram, cm^2/gram	=	Total surface-area

18. Estimate the viable harvest yield (cells per cm^2) of the entire spinner microcarrier culture using this formula.

Total viable cells	÷	Total surface-area	=	Viable harvest yield, cells per cm^2

19. Cell harvest yield can be converted to cell per mL using this formula.

Viable harvest yield, cells per cm^2	×	Theoretical surface-area per unit volume cm^2 per mL	=	Viable harvest yield, cells per mL

NOTE The theoretical surface area per unit volume is the microcarrier concentration (cm^2/mL) used to initiate microcarrier culture. For example, the theoretical surface area per unit volume for a 200 mL culture run at 5 cm^2/mL is 5 cm^2.

4 Disposal Considerations

The information in this section contains generic advice and guidance.

4.1 Waste Treatment Methods

4.1.1 Product

Methods of disposal

The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should **not** be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction.

Hazardous waste

Within the present knowledge of the supplier, this product is **not** regarded as hazardous waste, as defined by EU Directive 2008/98/EC.

4.1.2 Packaging

Methods of disposal

The generation of waste should be avoided or minimized wherever possible. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible.

Special precautions

This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Avoid dispersal of spilt material and runoff and contact with soil, waterways, drains and sewers.

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The information and figures contained in these instructions correspond to the version date specified below.
Sartorius reserves the right to make changes to the technology, features, specifications and design of the equipment without notice.
Masculine or feminine forms are used to facilitate legibility in these instructions and always simultaneously denote all genders.

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