Instructions for Use

Small-Scale Screening Studies Using Six-Well Plates

2620885-000-01

SARTORIUS

Contents

1 Introduction

Small-scale screening experiments in static multi-well plate culture formats are useful starting points to assess microcarrier and cell compatibility early in the development phase. Static multi-plate culture formats are easy and economic tools owned by most cell culture labs and they can be used to assess cell compatibility with multiple microcarrier types under semi-throughput format. Not unexpectedly, cells often grow to the highest densities on microcarriers that provide the most compatible surface, allowing for efficient attachment and favorable growth.

The following protocol provides a framework for the process of screening and visual qualitative assessment of SoloHill® Microcarrier types for cell attachment, spreading and growth under static culture conditions. Since these experiments are performed under static conditions (vs. dynamic), a population of cells are likely to adhere to the plate surface in addition to the microcarrier, and for that reason, a quantitative evaluation is not recommended. This small-scale screening protocol will familiarize the investigator with microcarrier handling and visualization of cells on microcarriers.

Cell and microcarrier compatibility will be evaluated by visual observation of cell health on the microcarrier surface over the course of 24 – 72 hours. This protocol can serve as a precursor to experiments designed at the optimization of conditions in small-scale stirred vessels and subsequent transfer of developed protocols into larger bioreactor formats.

SoloHill® Microcarriers are offered in multiple standard size formats ranging from 10 g to 1000 g quantities. For preliminary microcarrier and cell compatibility screening studies, the starter kit format (SK102-1521B) offers a portfolio of microcarrier types in 10-gram quantities. Refer Table 1 for a list of SoloHill® Microcarrier types and their properties.

Microcarrier Type	Relative Density Range	Size (microns)	Surface Area (cm^2/g)	Surface Charge (Yes | No)	Number of microcarriers per gram
Collagen – cross-linked polystyrene coated with type 1 porcine collagen	1.022 – 1.030	125 – 212	360	No	4.6×10^5
	1.034 – 1.046	125 – 212	360	No	4.6×10^5
	1.022 – 1.030	90 – 150	480	No	1.0×10^6
Fact III – cross-linked polystyrene coated with type 1 porcine collagen, cationic charge	1.022 – 1.030	125 – 212	360	Yes	4.6×10^5
Plastic – cross-linked polystyrene	1.022 – 1.030	125 – 212	360	No	4.6×10^5
	1.034 – 1.046	125 – 212	360	No	4.6×10^5
Plastic Plus – cross-linked polystyrene, cationic charge	1.022 – 1.030	125 – 212	360	Yes	4.6×10^5
Star-Plus, modified polystyrene, cationic charge	1.022 – 1.030	125 – 212	360	Yes	4.6×10^5
Hillex® II modified polystyrene, cationic charge	1.080 – 1.150	160 – 200	515	Yes	5.5×10^5

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

2 Materials and Equipment

- Autoclavable container (60 – 125 mL) (i.e., VWR 16120-737, 16120-741)
- Deionized water (DI) source
- Sterile Serological pipettes (1 mL, 5 mL, 10 mL)
- 6 well non-treated tissue culture plates (i.e. VWR 15705-056)
- P1000 Pipettor
- P1000 Aerosol barrier tips (i.e. VWR 89217-470)
- Cell culture medium
- Sigmacote® (Optional) (MilliporeSigma SL2)
- Hemocytometer or cell counting device
- Bio-safety cabinet (BSC)
- Verified balance
- Autoclave
- Humidified Incubator (37°C and 5% CO_2)
- Microscope (optional: with fluorescent imaging capability)

3 Procedure

3.1 Microcarrier Stock Preparation

To generate the most accurate and reproducible experimental results, each sample should contain an equivalent microcarrier surface area, medium volume and number of cells. Since the surface area (SA)-to-mass ratio varies depending on the microcarrier type used (see Table 1), the mass required for each microcarrier type to maintain constant SA across multiple samples may vary.

1. To prepare 20 mL stock solutions, each at a 12.5 cm^2/mL microcarrier density, collect separate autoclavable containers for each of the desired microcarrier types.
2. Measure the appropriate microcarrier mass for each respective microcarrier type (Table 2) and transfer to the autoclavable container.

Microcarrier type (size)	Microcarrier mass (gram)
Collagen (125 – 212 µm)	0.69
Collagen (90 – 150 µm)	0.52
Fact III (125 – 212 µm)	0.69
Plastic (125 – 212 µm)	0.69
Plastic (90 – 150 µm)	0.52
Plastic Plus (125 – 212 µm)	0.69
Star-Plus (125 – 212 µm)	0.69
Hillex® II (160 – 200 µm)	0.49

Table 2: Quantity of microcarrier required to prepare a 20 mL stock solution at 12.5 cm^2/mL microcarrier density

NOTE To prepare a known volume of stock solution at a custom microcarrier concentration for a specific microcarrier type use the following formula:

Stock volume (mL)	×	Microcarrier density (cm^2/mL)	÷	Microcarrier SA per gram (cm^2/gram)	=	Microcarrier mass (gram)

3. Add 20 mL of DI water to the container containing microcarriers.
4. Loosen the container cap to allow for sufficient venting and autoclave microcarrier stock solution at 121°C for 30 minutes.

   NOTE Length and temperature of sterilization may vary for different concentrations and volumes of microcarrier stock solutions.

5. Transfer the autoclaved microcarrier stock solution container(s) to the BSC in a sterile manner.
6. Allow the stock solution to cool at room temperature (RT) in the BSC before tightening the cap on the autoclaved container.
7. If sterility and performance (as defined by the end user validation) are maintained, this stock solution may be used for up to 3 months.

3.2 Microcarrier and Medium Acclimation

An important parameter to consider when performing screening studies is the concentration of serum and other protein(s) in the medium formulation during the cell attachment phase (from 1 to 4 hours post-cell inoculation). The sensitivity of cell attachment to serum or other proteins is a cell-specific phenomenon. For some cell types, serum or other proteins enables cell attachment to microcarriers and attachment is completely independent of protein concentration. However, for other cell types, serum or other proteins act as an inhibitor of attachment at elevated concentrations and manipulating the concentration of these components may be beneficial in optimizing cell attachment on microcarriers.

For serum-containing cultures, it is a good strategy to perform screening under conditions containing the desired serum concentration and under a reduced-serum environment. As a general guideline, low serum or protein concentration for attachment is typically between 0.05% and 0.5%.

Complete serum or supplement-free conditions are not generally recommended, as the serum | supplement contains factors that can enhance cell attachment as well as provide a protective benefit against any cell stress. Upon satisfactory cell attachment, serum or protein concentrations can be brought to the desired final concentration for the remainder of the culture duration. A similar strategy may be used for serum-free or defined medium formulations, where the supplement is provided in stand-alone format.

1. Aseptically transfer sterile microcarrier stock containers and well plates to the BSC.
2. Resuspend microcarriers in the stock bottle using a serological pipet.
3. Transfer 1 mL of stock solution per well in duplicates and allow microcarriers to settle at the bottom of the well.

   NOTE Use a different pipette for each of the individual microcarrier types. Ensure the stock solution is well-mixed before withdrawing a sample for the plate.

4. Aseptically transfer P1000 pipettor and sterile tips in the BSC.
5. Remove DI water from the sample well using P1000 pipettor.
   - Position the pipette tip close to the periphery of the well for support and wedge the tip to the bottom of the well containing microcarrier sample.
   - Slowly release the plunger and withdraw DI water from the sample well.

NOTE Be sure not to remove a significant number of microcarriers at this step. If a large amount of microcarriers are removed, add DI water back to the sample plate and repeat the process.

— Add 2 mL of cell culture medium to each sample well and re-distribute microcarriers by mixing plate in a side-to-side and up-to-down motion.

NOTE If cell attachment is being performed at low protein | serum concentration, add low serum | protein supplemented medium 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.

— Incubate the sample plate in the incubator at the desired temperature and CO_2 set points for at least 20 minutes.

3.3 Cell Addition

To obtain a uniform distribution of cells among microcarriers, it is essential to generate a robust, single-cell suspension that is free of aggregates and clumps. The following process is written as a guideline for seeding two 6-well plates at a seeding density of 2×10^4 cells per cm^2.

The optimal seeding density may vary from cell to cell and should be determined empirically for relevant processes. Typically, microcarrier cultures may be seeded at a density equivalent to that used to seed planar culture.

However, it is not recommended to seed microcarrier (MC) cultures at a seeding density lower than 3×10^3 cells per cm^2. When optimal cell attachment conditions are employed, a seeding density of 3×10^3 cells per cm^2 ensures that there are enough cells present to allow the microcarrier population to become occupied with at least one cell.

1. Calculate the total number of cells required to seed two 6-well plates at a 2×10^4 cells per cm^2 seeding density.

Number of wells		Volume of MC stock solution per well, mL		MC stock concentration cm^2/mL		Cell seed density cells /cm^2		Total number of cells required
12	×	1	×	12.5	×	2×10^4	=	3×10^6

2. Calculate the volume of cell suspension required to seed two 6-well plates.

Total cells required	÷	Cell suspension concentration cells/mL	=	Volume of cell suspension required, mL

3. Gently mix the cell suspension using a serological pipette and aliquot the required volume of cell suspension in the sterile conical-bottom tube.
4. Centrifuge cell suspension at desired speed & temperature setting, remove supernatant and resuspend cells in 6 mL cell culture medium to achieve a cell suspension concentration of 5×10^5 cells per mL.

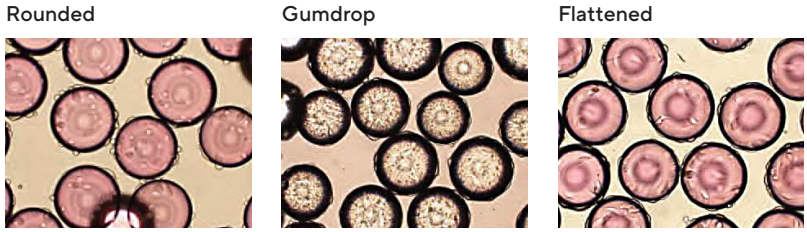
   NOTE If cell attachment is being performed at low protein | serum concentration, resuspend cells into low protein | serum supplemented medium. If cell attachment is being performed at full protein | serum concentration levels, resuspend cells in the growth medium. Gently resuspend cell pellet and avoid forming excessive bubbles to minimize shear stress on cells.

5. Remove well plates from the incubator and place them in the BSC.
6. Add 0.5 mL of the well-mixed cell suspension to each well and thoroughly mix plates in a side-to-side and up-and-down motion.
7. Transfer plates to the incubator and mix in side-to-side and up-and-down motion every 15 minutes for the first hour.

   NOTE Plates may be mixed longer than 1 hour if attachment kinetics are slower than anticipated.

3.4 Sample Analysis

1. Cell attachment and spreading can be observed using an inverted light microscope at various time intervals and qualitative assessments can be performed as early as 30 minutes post cell seeding. Cells can be visualized on the edges or circumference of the microcarriers as rounded (initial attachment phase), “gumdrop-shaped” (early spreading), or flattened (completely spread).



The ability to visualize cell spreading on microcarriers is dependent upon cell phenotypic characteristics. For example, fibroblast-like cells tend to exhibit a more flattened morphology than epithelial cells when spread on surfaces and may be more difficult to visualize.

Since static microcarrier culture samples are mixed intermittently, cell attachment to microcarriers can be uneven. For that reason, when evaluating the performance of each sample, it is more important to consider the percentage of microcarrier occupied by cells and morphology of the attached cells. A general benchmark for good performance is 75 – 90% of microcarrier occupancy (at least one cell per microcarrier) within the first one to four hours of incubation. Cell and microcarrier conditions that perform well by this criterion should be further evaluated in small-scale stirred vessels which allow for better control over cell attachment and growth uniformity.

3.5 Multiple techniques can be used to enhance visualization of cells on microcarriers

- Fluorescence microscopy (pre-loading cells with fluorescent dyes prior to seeding)
- DAPI staining after fixation with 4% paraformaldehyde
- Acridine orange
- Direct visualisation by phase microscopy
- Cell tracker (20 mg/mL phenol-red) staining for Hillex® II series

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.

Sartorius Stedim Biotech GmbH
August-Spindler-Strasse 11
37079 Goettingen, Germany

Phone: +49 551 308 0
www.sartorius.com

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.

Last updated:

08 | 2021

MB | DIR: 2620885-000-01