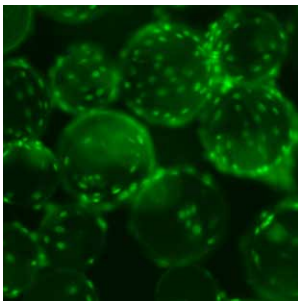
Corning® Microcarrier General Use Protocol

CORNING



This protocol is meant as a starting point for user optimization of microcarrier culture, specifically for use with Corning Microcarriers.

Suggested Materials

- Corning Synthemax II microcarriers (Corning Cat. No. 3781) or Corning Enhanced Attachment Microcarriers (Corning Cat. No. 3779)
- Sterile cell culture grade water (Corning cellgro® Cat. No. 25-055-CM)
- Disposable spinner flask
  - 125 mL (Corning Cat. No. 3152)
  - 500 mL (Corning Cat. No. 3153)
  - 1L (Corning Cat. No. 3561)
  - 3L (Corning Cat. No. 3563)
- Slow speed stirrer
- Cell culture medium
- Calcein AM (Falcon® Cat. No. 354216)
- Cell lysis buffers (Eppendorf® Cat. No. P0820-5190 and P0820-5180)
- 40 µM cell strainer (Falcon Cat. No. 352340)
- Multiple well microplates
- DPBS or HBSS (Corning cellgro Cat. No. 21-031-CM or 21-022-CM)
- Trypsin (Corning cellgro Cat. No. 25-052-CV) or other cell dissociation solution

Bead Preparation

1. Aseptically pour the entire contents of the 10 g vial of microcarriers to a sterile container that can hold at least 100 mL of volume.
2. Rinse vial with 10 mL of sterile water and add rinse solution to dry microcarriers.
3. Add additional sterile water to microcarriers to achieve a final volume of 100 mL for a final concentration of 100 mg/mL (36 cm^2/mL).

   Note: If using the full contents of the microcarrier vial (10 g), you may reconstitute directly in culture medium.

Cell Seeding

1. Transfer the desired amount of beads to a suitable sterile container such as a storage bottle, centrifuge tube or spinner flask. Unused resuspended beads should be stored at 4°C.
2. Once beads have settled, the water may be aseptically removed by careful aspiration or pipetting. Care should be taken not to aspirate microcarriers.

3. Reconstitute the microcarriers in media or media containing cell inoculum. *Recommendation*: Seed using the lowest possible volume in which stirring can still occur. This will give the cells a better chance for attachment. Example: 30 mL in a Corning® 125 mL spinner vessel, 100 mL in a Corning 500 mL vessel, 50 mL in a Corning 1L vessel, and 150 mL in a Corning 3L vessel.
4. Place the spinner flask in the incubator to allow for cell attachment.

   Note: There are different conditions for the initial cell attachment that will vary depending on the application and should be optimized by the user. Factors to consider are:

   - Amount of time for cell attachment
     Example: 3 hours to overnight
   - Volume used during cell attachment
     Example: 28 mL in 125 mL spinner flask
   - Stir speed and occurrence (constant or intermittent)
     Example: 15 rpm for 2 minutes every 30 minutes

Once cells have attached, the volume in the spinner flask can be increased for the duration of the culture period.

Recommendation: Increase the volume to approximately half the stated volume of the vessel (example: 60 mL in a 125 mL vessel).

Note: Stir speed and occurrence (e.g., constant vs. intermittent) may need to be optimized for each cell type, application and amount of microcarriers used.

Cell Visualization

For cell visualization, take a sample of the culture solution and transfer to a well of one 6 or 24 well multiple well cell culture plate. Visualizing the cells on the microcarriers may be difficult; therefore we recommend staining the cells with a live cell fluorescent stain such as calcein AM to aid in visualizing the cells and their overall confluence.

Cell Counting Without the Need for Viable Single Cells

If a viable single cell suspension is not required, cells may be lysed for easy enumeration.

1. Remove an aliquot of the microcarrier culture and transfer to a multiple well microplate (example: 6 well cell culture plate).
2. Allow microcarriers to settle and gently remove cell culture media.
3. Wash microcarriers 2 times with DPBS.
4. Add 1 mL of cell lysis buffer (Reagent A) and observe lysis under microscope (typically less than 5 minutes).
5. Separate the lysed cells from microcarriers by using a cell strainer and a 6 well plate or 50 mL centrifuge tube.
6. Use 1 mL of Reagent B of cell lysis buffer to wash source well and the filter and then collect into the same well from step 5.
7. Gently swirl the plate to mix reagents and observe cell nuclei under microscope.

   Note: Nuclei may be stained with a fluorescent dye such as Hoechst for easy counting via a fluorescent microscope.

Cell Harvesting

1. Separate media from microcarriers by methods such as centrifugation or gravity.
2. Wash beads 1 to 2 times with DPBS or HBSS without calcium or magnesium using same method from step 1 of harvest.
3. Add appropriate volume of trypsin or desired detachment solution to microcarriers containing cells.

   Note: Some pipetting or incubation at 37°C may be required to aid in cell detachment.
4. Once cells are detached, the harvesting solution should be quenched or diluted to prevent cell damage.
5. Detached cells can be separated from the microcarriers by filtering the cells through a 40 µM cell strainer. Multiple DPBS rinses may be required to ensure all cells are collected.

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