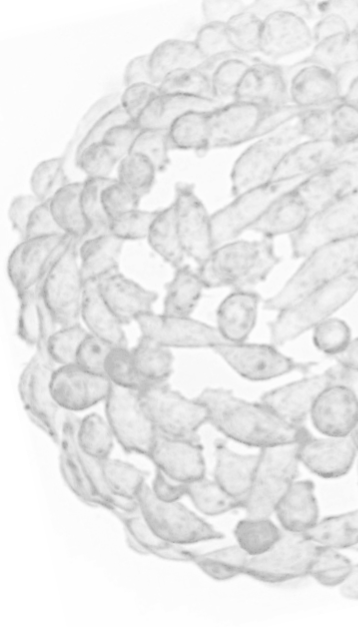

Adhesion Assay Kit

This protocol is a quantitative method for evaluating adhesion to the GEM. A typical setup is type tested 50,000 cells and 50 uL of GEM run in triplicate. Keep in mind adhesion preference can change with the type, concentration or presence of serum. For a stringent test, compare adhesion with and without serum present.

- Multi-well low attachment or poly-HEMA coated dish
  This protocol is written for a 24 well dish. Other formats may require the volumes be adjusted.
- 50 ml of culture medium
- PBS
- Accutase
- 50 uL of GEM per well you wish to test
- 50,000 cells per well you wish to test

1) Add 450 uL of medium to each well you intend to use.
2) Appropriately label a 24 well low attachment dish and add 50 uL of the appropriate GEM to each well. A well free of GEMs provides a nice control for cell viability.
3) Create a cell suspension of 1.5 million cells in 15 mL of medium
4) Dispense 500 uL of cell suspension into each well. Swirl gently. Observe with a microscope.
5) Place in a tissue culture incubator at the appropriate temperature and CO_2. Adhesion times can range from 30 minutes to over 24 hours. As a rule of thumb, cells that are

slow to adhere in 2D will also adhere more slowly to the GEM. The vast majority of cells are attached within 4 hours.

6) Swirl the plate firmly to re-suspend the GEMs. Using a magnet, hold the GEMs in the bottom of the well. Remove 250uL of supernatant to a micro centrifuge tube. Count the number of cells still in suspension and calculate C_{NA}.

Calculate:
Cell Count x 4 = C_{NA} or Number of Non-adhered Cells

7) Using a magnet, hold the GEMs in the bottom of the well and carefully aspirate off the remaining media. Add 250 uL Accutase per well.
8) Wait 10 min at room temp for cells to come off the GEMs.
9) Swirl plate. Using a magnet, hold the GEMs in the bottom of the well and remove 125 uL and count. Calculate adhesion efficiency.

Calculate:
Cell Count x 2 = C_G or Number on GEMs
For each well:
C_{NA} + C_G = TVC = Total Viable Cells
C_{NA} / TVC = Percent Cells Non-adhered
C_G / TVC = Percent Cells Adhered
Loading Efficiency is then:
C_G / C_{NA} = Loading Efficiency when C_{NA} > 0

Additional Tips

- Use your traditional culture substrate as a positive control for the assay with and without serum.
- Count cells as you would traditionally applying the same dilutions and corrections.
- For increased precision in step 9, remove the remaining solution and calculate the total volume of the well. Correct the counts appropriately.