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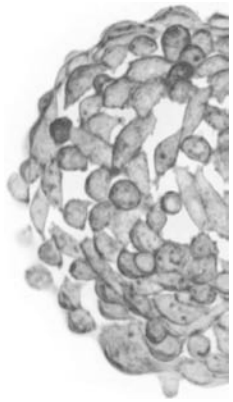

Protocol: Adhesion Assay Kit

This protocol is a quantitative method for evaluating adhesion to the GEM. A typical setup tests 100,000 cells with 50µL 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.

Materials Needed

- 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. The following table suggests volumes for different formats.

Vessel	GEM	Cells	Well Volume
6 cm dish	250µL	500,000	5mL
10 cm dish	500µL	1,000,000	10mL
6-well plate	150µL	300,000	3mL
24-well plate	50µL	100,000	1mL
96-well plate	5µL	10,000	100µL

- 50mL of culture medium
- PBS
- Accutase
- 50µL of GEM per well you wish to test
- 100,000 cells per well you wish to test

1. Wash the GEM once with media to remove the storage buffer (2.5mM $CaCl_2$, 10mM HEPES).
2. Add 450µL of medium to each well you intend to use.
3. Appropriately label a 24-well low attachment dish and add 50uL of the appropriate GEM to each well. A well free of GEM provides a nice control for cell viability.
4. Create a cell suspension of 3 million cells in 15mL of medium.
5. Dispense 500µL of cell suspension into each well. Swirl gently. Observe with a microscope.
6. Place in a tissue culture incubator at the appropriate temperature and CO2. 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.

7. Swirl the plate firmly to re-suspend the GEMs. Using a magnet, hold the GEMs in the bottom of the well. Remove 250µL of supernatant to a micro centrifuge tube. Count the number of cells still in suspension and calculate CNA.

Calculate:

Cell Count x 4 = CNA or Number of Non-adhered Cells

8. Using a magnet, hold the GEM in the bottom of the well and carefully aspirate off the remaining media. Add 250µL Accutase per well.

9. Wait for 10 minutes at room temperature for cells to come off the GEM.

10. Swirl plate. Using a magnet, hold the GEM in the bottom of the well and remove 125µL and count. Calculate adhesion efficiency.

Calculate:

Cell Count x 2 = CG or Number on GEMs

For each well:

CNA + CG = TVC = Total Viable Cells

CNA / TVC = Percent Cells Non-adhered

CG / TVC = Percent Cells Adhered

Loading Efficiency is then:

CG / CNA = Loading Efficiency when CNA > 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 while applying the same dilutions and corrections.
- For increased precision in step 7, remove the remaining solution and calculate the total volume of the well. Correct the counts appropriately.