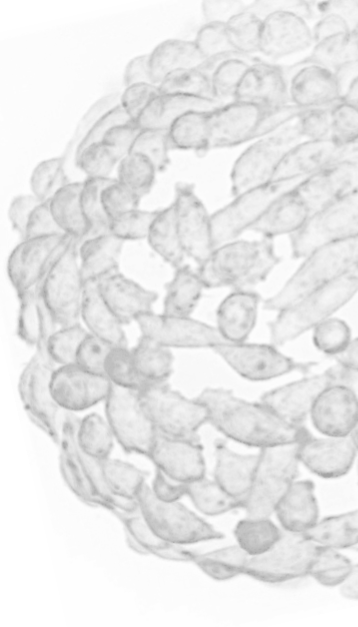

Culturing Cells Using the GEM

Questions? Suggestions? We want to help! Please contact us at:

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The following protocol describes how to: 1) load cells onto the GEM, 2) maintain cells on the GEM and 3) collect and passage cells with the GEM. These protocols are intended for use with any of the petri dish or multi-well plate GEM products.

Materials:
-GEM substrate of choice
-Cells
-Media
-Ultra low adhesion dish or multi-well plate

Suggested volumes per well:

Vessel	GEM	Cells	Media
6 cm dish	100 uL	100,000	5 mL
10 cm dish	500 uL	500,000	10 mL
6-well plate	100 uL	100,000	3 mL
24-well plate	50 uL	50,000	500 uL
96-well plate	5 uL	5,000	50 uL

Loading the GEM:

1) Wash the GEM once with media to remove the storage buffer (2.5 mM $CaCl_2$, 10 mM HEPES).
2) Add the media to the culture vessel.
3) Add GEMs to the media.
4) Prepare a cell suspension. Accutase can collect cells from plastic as well as GEMs.

Have sensitive cells? Want better cell viability? Be sure to read the “Additional Tips” below.

5) Add cells to the media/GEM preparations.
6) Shake gently and place in a tissue culture incubator.
7) Check monitor for cell adhesion. Once cells have adhered, place the BioMagnet above the cultures to pull the GEMs and cells to the surface.

Maintaining Cells on the GEM:

1) Media exchange is done in the same manner as traditional cell culture using an indicator such as phenol red.
2) Using the Cube Magnet, hold the GEMs in the corner of the well. Gently aspirate the used media leaving 1/4 to 1/3. Leaving some used media will avoid shocking the cells.
3) Add the fresh warm media.

Collecting Cells from the GEM:

1) Using the Cube Magnet, hold the GEMs in the corner of the well. Remove as much media as possible. Wash once with PBS.
2) Using the Cube Magnet, hold the GEMs in the corner of the well. Aspirate the PBS and add Accutase. Use the volumes listed above for media.
3) Gently agitate at room temperature. Observe detachment with a microscope. Note the time required for future work.
4) Using the Cube Magnet, hold the GEMs in the corner of the well. Aspirate your cell suspension.
5) To passage, take a portion of the collected cells and load onto new GEMs.

Additional Tips

- Use Accutase to prepare your cell suspension for maximum viability.

- One hour before passaging cells apply ROCK inhibitor to a final concentration of 5-10 mM. Passage the cells as usual. This treatment will increase the viability of primary and stem cells during passaging.
- Cells on GEM will begin to aggregate over time. Using the 10 cm ULA dish on a rocker in the incubator will help decrease aggregation.
- Serum concentrations can be reduced in GEM culture because there is no need to adsorb protein to the culture surface. Serum can be removed all together but a proper serum free media must be used.