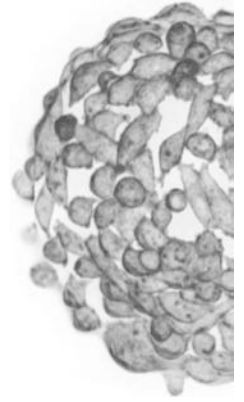


Global Cell Solutions Culture. Simplified.



Protocol: 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
- 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	Well Volume
6 cm dish	250 μ L	500,000	5mL
10 cm dish	500 μ L	1,000,000	10mL
6-well plate	150 μ L	300,000	3mL
24-well plate	50 μ L	100,000	1mL
96-well plate	5 μ L	10,000	100 μ L

1. Wash the GEM once with media to remove the storage buffer (2.5mM CaCl₂, 10mM HEPES).
2. Add the media to the culture vessel.
3. Add GEM to the media.
4. Prepare a cell suspension. Accutase can collect cells from plastic as well as from GEM.
5. Add cells to the media/GEM preparations.

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6. Shake gently and place in a tissue culture incubator.
7. Check and monitor for cell adhesion. Once cells have adhered place the BioMagnet above the cultures to pull the GEM 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 CubeMagnet, hold the GEM in the corner of the well. Gently aspirate the used media leaving a quarter to a third of the media. Leaving some used media will avoid shocking the cells.
3. Add the fresh warm media.

Collecting Cells from the GEM:

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

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 5-10mM. 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 10cm 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.