Application Notes

Culturing Cells on GEM™ in the Biolevitator™

CELL TYPE: **Immortalized Human Renal Proximal Tubule Cell (h PTC, HK-2)**
PROTOCOL: **Culture Without Conditioned Media**

Acknowledgements to Dr. Robin Felder and Dr. John Gildea of the University of Virginia.

Media Needed[1]:

- **Type of Media: DMEM/F12**
- **Additional Supplements:**
  - **2% Serum;**
  - **36ng/ml Dexamethasone;**
  - **1X Penicillin / Streptomycin;**
  - **10ng/ml Epidermal Growth Factor (EGF);**
  - **1X Insulin Transferrin Selenium (ITS);**
  - **2ng/ml Triodothironine (T3);**
  - **2.5µg/ml Plasmocin**

Before you Begin:

- Wash and acclimate the GEM[2].
- Harvest 2 million h PTC, HK-2 cells. It is important to keep the volume of cell suspension minimal. It is ideal to keep the volume of cells to 1mL or preferably, less.
- Set the BioLevitator parameters for "Inoculation" and "Culture" based on the tables below.

TABLE 1. INOCULATION PARAMETERS	
Inoculation Time (hours)	4
Rotation	two way
Speed	75 RPM
Interval Time	2 minutes
Agitation Pause	40 minutes
Pause Between Rotation	0 seconds
Rotation Period	1 second

TABLE 2. CULTURE PARAMETERS	
Rotation	two way
Speed	75 RPM
Interval Time	∝
Agitation Pause	0 minutes
Pause Between Rotation	0 seconds
Rotation Period	1 second

Inoculation:

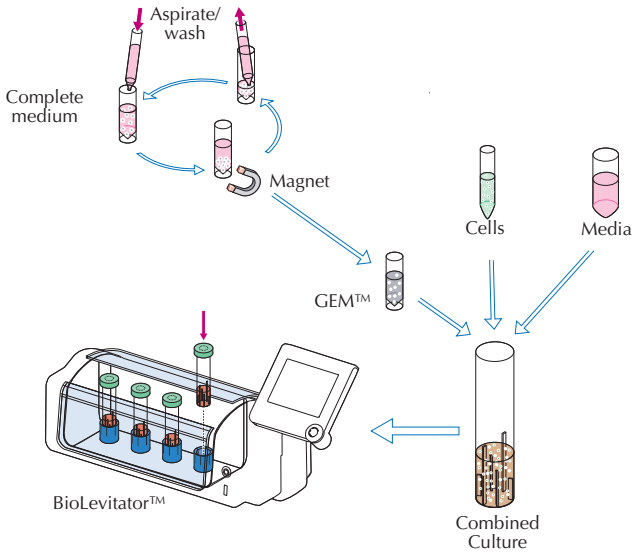
1. Turn the BioLevitator on and pre-warm it. Adjust the carbon dioxide on the BioLevitator to the appropriate levels and verify that the levels are accurate[3].
2. Add 30mL of fresh complete culture media to the tube.

Note: *Adjust the volume of fresh media added during inoculation to compensate for the volume of cells added in order to maintain a total inoculation volume of 30mL. You may exclude the volume of GEM (0.5mL) from this total.*

3. Invert the vial of washed GEM as needed to re-suspend the GEM substrate.
4. Quickly draw up *250µl*[4] of re-suspended GEM slurry in a pipette and dispense this into the BioLevitator tube.
5. Add the cell suspension containing approximately 2 million cells to the tube.
6. The total volume in the tube should now be approximately 30mL.

Note: *Order of addition is important! Always add cells last!*

7. Put the cap on the tube and place in the BioLevitator.

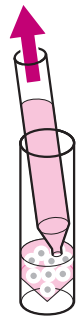
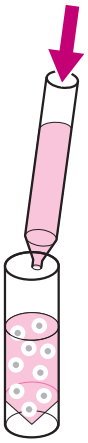


[1] For a complete list of materials needed, see Section 3. "Working with the GEM" in the manual "Culturing Cells on GEM using the BioLevitator" provided by Global Cell Solutions.
[2] See protocol in Section 3.1 "Preparing the GEM" in the manual "Culturing Cells on GEM using the BioLevitator" provided by Global Cell Solutions.
[3] See BioLevitator User Manual provided by Hamilton Company.
[4] Volume is 50% packed slurry.

Feeding the BioLevitator Cultures

Feed the cultures according to the Table 3. Take cell counts as necessary throughout the culture period.

TABLE 3 SCHEDULE FOR FEEDING THE CULTURES

Experimental Day		Volume of Media Removed (mL)		Addition of Fresh Media (mL)		Rotation Speed	Total Volume After Feeding
Inoculation Day	AM	0mL	 *Aspirate volume of media*	30mL	 *Add fresh media to the tube*	75RPM	30mL
	PM	0mL		0mL			
Day 1	AM	0mL		0mL		75RPM	30mL
	PM	0mL		0mL			
Day 2	AM	25mL		25mL		75RPM	30mL
	PM	0mL		0mL			
Day 3	AM	0mL		0mL		75RPM	30mL
	PM	0mL		0mL			
Day 4	AM	0mL		0mL		75RPM	30mL
	PM	0mL		0mL			
Day 5	AM	0mL		0mL		75RPM	30mL
	PM	0mL		0mL			
Day 6	AM	25mL		25mL		75RPM	30mL
	PM	0mL		0mL			

Note: *After Day 6, you may choose to harvest your cells, or you may continue the culture. As the culture expands, the limiting factor becomes the rate of feeding. It is advisable to split your cultures into multiple tubes in order to continue the culture expansion.*

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