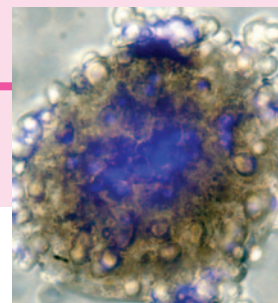


Culturing Cells on GEM™ in the Biolevitator™

CELL TYPE: **Human Embryonic Kidney 293 (HEK 293)**

PROTOCOL: **Culture Without Conditioned Media**



20X fluorescent overlaid image stack of HEK 293 cells growing on GEM™ substrate stained with 0.1mg/mL Hoechst Stain

Media Needed¹:

- **Type of Media:** DMEM/F12
- **Additional Supplements:**
 - 15mM Hepes
 - 10% Fetal Bovine Serum
 - 1% Penicillin/ Streptomycin

Before you Begin:

- Wash and acclimate the GEM².
- Harvest 6 million HEK 293 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 BioLevigator parameters for “Inoculation” and “Culture” based on the tables below.

TABLE 1. INOCULATION PARAMETERS	
Inoculation Time (hours)	4
Rotation	two way
Speed	60 RPM
Interval Time	2 minutes
Agitation Pause	40 minutes
Pause Between Rotation	0 seconds
Rotation Period	1 second

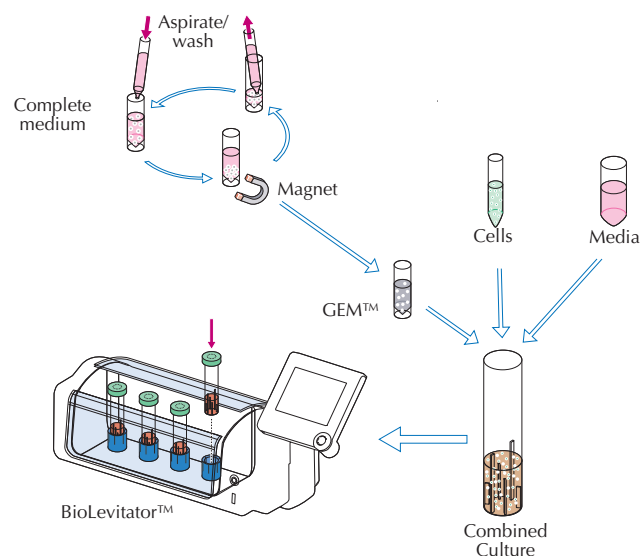
TABLE 2. CULTURE PARAMETERS	
Rotation	two way
Speed	60 RPM
Interval Time	5 days
Agitation Pause	0 minutes
Pause Between Rotation	0 seconds
Rotation Period	1 second

Inoculation:

1. Turn the BioLevigator on and pre-warm it. Adjust the carbon dioxide on the BioLevigator to the appropriate levels and verify that the levels are accurate³.
2. Add 10mL 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 10mL. 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 0.5mL⁴ of re-suspended GEM slurry in a pipette and dispense this into the BioLevigator tube.
5. Add the cell suspension containing approximately 6 million cells to the tube.
6. The total volume in the tube should now be approximately 10mL.

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

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



¹ For a complete list of materials needed, see Section 3. “Working with the GEM” in the manual “Culturing Cells on GEM using the BioLevigator” provided by Global Cell Solutions.

² See protocol in Section 3.1 “Preparing the GEM” in the manual “Culturing Cells on GEM using the BioLevigator” provided by Global Cell Solutions.

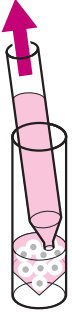
³ See BioLevigator User Manual provided by Hamilton Company.

⁴ Volume is 50% packed slurry.

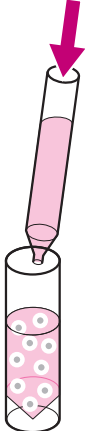
Feeding the BioLevigator Cultures

Feed the cultures according to the Table 3. Take cell counts as necessary throughout the culture period. Harvest the cultures on Day 5.

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	60RPM	10mL
	PM	0mL		
Day 1	AM	0mL	60RPM	10mL
	PM	0mL		
Day 2	AM	0mL	70RPM	20mL
	PM	0mL		
Day 3	AM	5mL	75RPM	40mL
	PM	0mL		
Day 4	AM	35mL	80RPM	40mL
	PM	0mL		
Day 5	AM	35mL	80RPM	40mL
	PM	35mL		



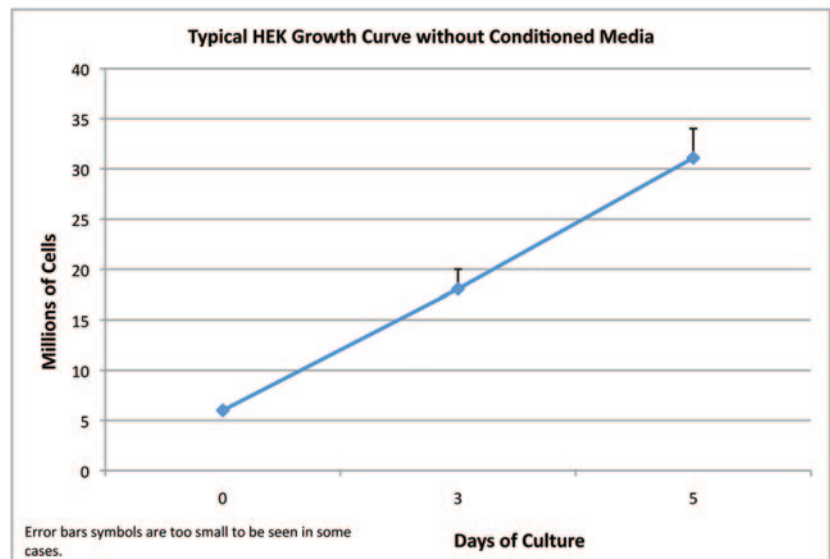
Aspirate volume of media



Add fresh media to the tube

Note: After Day 5, 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.

A typical growth curve generated from HEK 293 cells in a BioLevigator™.



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