ABCEPTA CUSTOM SERVICES:

Cell Culture Protocol

Thawing Cells

1. Place frozen cells in 37 ℃ water bath for approximately 2 minutes or until cells are thawed.
2. Place cells into MEM media (which should be at room temperature) + 10% FBS for 10 minutes.
3. Centrifuge the cells at 1200 rpm for 5 minutes at room temperature.
4. Resuspend the cells to a final concentration of 10^5 cells/mL.

Culturing the Cells

1. Place ~2.5 $\times 10^6$ cells per culture flask (25 mL of 1 $\times 10^5$ resuspended cells).
2. Close the flask lid, but not too tightly.
3. Place the flask with cells in the incubator at 37 ℃ with 5% CO_2.
4. Check the flasks daily for changes in media color and/or monolayer.

Changing the Cell Culture Media

1. Always leave one flask alone and feed the following day for fear of contamination problems.
2. Cultures should be fed every 2/3 days – once the media starts to change color.
3. Take the culture flasks out of the incubator and place in the laminar flow hood.
4. Remove 50% to 75% of current media from the flask. Replace the amount taken with room temperature MEM media (10% FBS).
5. Place the flask back into the incubator and record the information on the data sheet.

Subculturing Cells

1. Remove present culture media.
2. Add 10 mL of 0.025%-0.25% trypsin, and let the cells sit for 10 minutes at room temperature. It may be necessary to bang the culture flasks on the hood counter to remove any “sticky” cells from the flask surface.
3. Immediately after the 10 minutes have passed – add room temp MEM media (10%FBS) to inactivate the trypsin.
4. Adjust the cell concentration to 1 x 10^5 cells per mL.
5. Add 2.5 $\times 10^6$ cells per culture flask (25 mL of 1 $\times 10^5$ resuspended cells).
6. Close the flask lid, but leave slightly lose.
7. Place culture flasks back into the incubator and check daily for media changes/monolayer formation.
8. Record information on data sheet.

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