Protocol for Passaging HIEC-6 cells

Materials

Pasteur Pipettes
Flasks (T25 or T75)
Water Bath at 37C
Stripettes (2 mL 5 mL, 10 mL)
PBS
0.25% Trypsin EDTA (ThermoFisher Scientific catalog #: 25200072)
Opti-MEM media (can be stored up to 2 weeks at 4C):

- 10 ng/mL EGF (ThermoFisher Scientific catalog #: PHG0315)
- Opti-MEM supplemented with HEPES and Glutamax (Gibco catalog #: 51985034)
- 10% FBS
- *I have cultured the cells with both 4% FBS (as recommended on ATCC website) and 10% FBS in parallel, but I did not notice a difference.*

37C Incubator with 5% CO_2

1) Preparation
    a. Turn on 37C water bath. Let temperature equilibrate to 37°C (approx. 15 mins).
    b. Place the PBS and HIEC-6 media in the 37°C water bath. Allow temperature to equilibrate to 37°C (approx. 15 mins).
    c. Allow Trypsin solution to warm to room temperature (15 mins).
    d. Turn on UV light in biosafety cabinet (approx. 15 mins)
    e. After 15 mins, turn off UV light in the biosafety cabinet. Turn on the fluorescent light and blower. Open the biosafety cabinet and spray down surface with ethanol (liberally!).
    f. Transfer all materials to the hood (stripettes, PBS, Trypsin, Opti-MEM, new flasks)
2) Trypsinize cells
    a. Remove flask of HIEC-6 cells from the 37°C incubator. Check with inverted microscope to make sure that flask is between 90-100% confluent.
    b. In biosafety cabinet, remove media with a Pasteur pipette and vacuum pump.
    c. Rinse cells by adding 2-5 mL of pre-warmed PBS to the flask. Remove with a Pasteur pipette.
    d. Add 0.5 – 1mL (0.5 mL for T25 flask, 1 mL for T75 flask) of room temperature trypsin-EDTA. Return flask to the 37C incubator for 5 minutes. Check for evidence of detachment (cells will appear as "flakes"). *Note: HIEC-6 cells require longer to detach (compared to HeLa cells). I have not noticed any problems incubating them with Trypsin-EDTA for >5 mins.*
    e. Stop trypsinization by adding Opti-MEM (4.5 mL for T25 and 9 mL for T75). Pipette cells up and down 8-10 times to break up clumps.
3) Seed a new flask.
    a. For HeLa cells, we will use the following split ratios. Add the appropriate amount of cell suspension and media, and transfer to a new flask.

Split Ratio	mL cells /mL media for T25	mL cells /mL media for T75
1:4	1 mL + 4 mL	2 mL + 8 mL
1:5	0.8 mL + 4.2 mL	1.6 mL + 8.4 mL
1:6	0.7 mL + 4.3 mL	1.4 mL + 8.6 mL
1:8	0.6 mL + 4.4 mL	1.2 mL + 8.8 mL
1:10	0.5 mL + 4.5 mL	1.0 mL + 9 mL

    b. Label flask! (cell line, date, passage #, initials) and return to the 37°C incubator. *These cells do not do well if the split ratio is too high. 1:4 or 1:5 is optimum.*

For Generating Freezer Stocks

1) Retrieve pre-chilled isopropanol cell culture freezing container from the -80°C freezer.
2) Follow steps 1-2d above.
3) Following tryspinization, resuspend cells in 9 mL of freezing medium containing 90% FBS and 10% DMSO.
4) Aliquot 1 mL of re-suspended cells into each cryovial.
5) Place cryovials containing cells into the pre-chilled cell culture freezer and replace into the -80°C freezer.
6) Keep cells at -80°C overnight, and move to the liquid nitrogen tank the next day (tower 4, box 1).