HPDE6-E6E7 cell culture conditions

From: Duke/UNC/UTA/EBI ENCODE group
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Source of cells: Dr. Ming-Sound Tsao, Ontario Cancer Institute. Citation describing these cells: Furukawa, Duguid, Rosenberg, Viallet, Galloway, and Tsao, "Long-Term Culture and Immortalization of Epithelial Cells from Normal Adult Human Pancreatic Duts Transfected by the E6E7 Gene of Human Papiloma Virus 16". (1996) American Journal of Pathology, Vol 148, No.6

Lineage of cells: normal human pancreatic duct cells immortalized with E6E7 gene of HPV

Donor information: female (63yo)

Karyotype: Unknown

Medium:
-Keratinocyte Serum Free Media: KSF (Invitrogen #17005-042). This comes with 1 tube of bovine pituitary extract (25mg) and 1 tube of EGF (2.5ug)
-Reagent pack (Lonza #CC-5034)
Hepes, trypsin, and trypsin neutralization solution

Procedure:
1. Frozen cells should be thawed into a 60 mm2 flask containing 6 ml of medium and incubated @37C, 5% C02 and allowed to attach; change the media at the second day. Let the cells grow to fill out the dish, then split.

2. Trypsinize with 0.05% trypsin. Split 1:5.
(a) Remove the media
(b) rinse the cells with 1 X PBS or Hepes solution that comes with reagent pack.
(c) add 1 mL 0.05% trypsin to cells, place at 10 minutes in 37 degree incubator. Check every 3 minutes and tap plate to loosen cells. Cells won't detach easily.
(d) add 1 mL of trypsin neutralization solution, pipet up and down several times.
(e) transfer cells to 15ml conical tube, centrifuge 4 minutes at 1000rpm.
(f) aspirate off supernatant, resuspend cells in 10mL KSF.
(g) count cells (9 million/T75 flask when confluent).
(h) plate at desired density.

3. Change the media every two days. For production, we grow these cells either in 15 cm dishes or T175 flasks.
(a) check the culture every two days; (ii) confluence is less than 75%. Otherwise, change media and split cells.

4. Grow to 75% confluence before harvesting

Comments:
These cells are slow growing and should have media changed every 2-3 days. They are very sensitive to trypsin, hence the centrifugation step. Do not trypsinize and split, the cells will not attach.