

IMEC Maintenance (Culturing and Misc.)

Background Information:

- IMEC (Immortalized Mammary Epithelial Cells) were immortalized in Dr. Myles Brown's laboratory at the Dana Farber Cancer Institute by stably transducing them with a telomerase gene expression vector. Therefore, these cells are not transformed (they presumably have not accumulated associated genetic alterations, although p16 is likely to be hypermethylated).
- IMECs were immortalized by introducing a telomerase gene expression vector and puromycin was used to select for transductants. So, these cells are resistant to 0.5ug/ml Puro and should generally be cultured in the presence of 0.5ug/ml Puro.

Culturing:

- IMECs must be cultured in MEGM media (Bio*Whittaker, Cat# CC-3051)[supplemented with bovine pituitary extract (supplied by vendor)].
- Cultures that are around 70-80% confluency should be split 1:4 into fresh medium.
- Two days after splitting, media should be changed.
- Two days after changing media, cells should be split again.
- Cells must be trypsinized using the trypsin pack from Bio*Whittaker (Cat# CC-5034) (the trypsin we use for tumor cells will kill these cells in no time).

Trypsinization:

Cells must be trypsinized using the Trypsin Pack from Bio*Whittaker (Cat# CC-5034).

Splitting (for 100mm plate):

- Take off media and wash one time with 6ml HBBS [from Trypsin Pack (Bio*Whittaker)].
- Add 6ml Trypsin [from Trypsin Pack (Bio*Whittaker)].
- Incubate for approximately 5-10 minutes making sure that the trypsin always covers the entire plate (be patient here, it takes at least 5 minutes) [Note: trypsin should be aliquoted after the first time it is thawed because it becomes inactive very quickly (within a week) if it is stored at 4°C. With weak trypsin, you'll kill your cells before they come off of the plate].
- After 5-7 minutes, pipette trypsin up and then squirt back onto plate. If cells start coming off quite readily, then the trypsinization is complete. In this case pipette up and down until most of the cells have come up. Then add 6ml of TNS Solution [from Trypsin Pack (Bio*Whittaker)] to neutralize trypsin.
- Spin cells down, resuspend in desired amount of MEGM and replate (Important: it is crucial to spin cells down to get rid of trypsin. I didn't do this once and the cells never adhered to the plate and they died!).

Note: Cells are fragile so we always prewarm media and trypsinization reagents. Trypsin is weak, so its best to thaw an aliquot every time you need it and don't warm for very long before using.

IMEC Misc. Info

G418 Sensitivity = 50 ug/ml

Hygro Sensitivity = 25ug/ml