Gourronc and Klingelhutz

Growing keratinocytes, cervical, and tonsillar epithelial cells in K-SFM.

KSFM stands for Keratinocyte Serum Free Media. It's a media specifically used to grow keratinocytes. Fibroblasts do not grow in KSFM. This medium is not used when Feeders are mixed with Keratinocytes.

Commercially available K-SFM media needs to be supplemented with BPE, EGF and Penn/Step solutions. The catalog number for the media and supplements is 17005-042.

Preparing media

For one bottle of K-SFM (500ml). Note: You do not add all the EGF and BPE!

Add	0.16 ng/ml (0.08 ug per 500 ml)	EGF (each lot has a different concentration, written on the tube). Store the remaining EGF in the -20 in a box labelled "EGF".
	25 ug/ml (12.5 mg per 500 ml)	BPE (each lot has a different concentration, written on the tube)
	5 ml	(100X) Pen/Strep

Do not keep media for more than 1 month past adding supplements. Do not freeze and thaw media.
Change media every other day. The cells will not do well if they are left over the weekend without a media change or if they are left to grow to greater than 95% confluency.

Incubator settings: Normal 5% CO_2 and 37^{o}C with humidity

How to pass cells growing in K-SFM

1. Cells are passed when they are 70-90% confluent
2. Aspirate media.
3. Wash plate with (1X) PBS (PBS without calcium or magnesium).
4. Add 4 ml (0.05%) Trypsin/EDTA.**MAKE SURE IT IS 0.05%!**
5. Incubate at 37^{o}C for 5 to 10 minutes. Tap plate and view under scope to see if cells have lifted.
6. Add 4 ml PBS-A containing 2% FBS to inactivate the trypsin. This is important!
   Pipette off attached cells. Transfer to a sterile 15ml tube.
7. Centrifuge at 1200rpm for 5min. It is important to spin out the FBS/trypsin solution.
8. Prepare new plates and label carefully: Cell Name, Passage Number (Be sure to increase passage number by 1), Date, Your initials.
9. Carefully aspirate supernatant taking care not to disturb the pellet.
10. Disperse pellet in K-SFM:
    For a 1:4 split, bring up cells in 8 ml, mix well. Add 2 ml dropwise over entire plate to 8mls K-SFM already in plate. Do not swirl.

Keratinocytes are usually split every 4 or 5 days and Primary Cells usually survive for 6 to 10 passages when growing in K-SFM.

Freezing and thawing cells in K-SFM

Freezing cells

K-SFM Freezing Media (100 ml)

10ml DMS0

15ml FBS

75ml K-SFM

1. Usually 3 vials are made per 80% confluent 10 cm plate. Label 3 cryo-vials: Include name of cell line or strain, passage number, date, and initials.
2. Prepare cells as described above up to #9. Disperse pellet in 4.5 ml K-SFM freezing media.
3. Aliquot 1.5 ml into each labeled cryovials.
4. Slow cool in a freezing container containing isopropanol or by using a styrofoam rack and progressively bringing down the temperature (30 minutes at 4°C, 1 hour at -20°C, overnight at -80, liquid nitrogen). Do not leave cells in -80 C for more than a week or they will begin to lose viability. After transferring to liquid nitrogen, log location in **AK freezer book**.

Thawing cells

1. Remove vial from liquid nitrogen and quickly place in 37 C water bath in a float. Erase notation from freezer book after taking notes on label and location. Since some nitrogen gas can enter the vial, they can pop or, rarely, explode. Take proper precautions (e.g. wear protective eye glasses)..
2. After liquid is thawed (Do not leave for more than 5 minutes) , bring to hood and transfer, using a 5 ml pipette, to 5 mls PBS in a 15 ml tube. This dilutes out the DMSO and FBS used in the freeze media. If this is not removed, the cells will differentiate and die. Mix gently with pipette.
3. Spin at 1,200 RPM in table top for 5 minutes.
4. Carefully aspirate media while tipping tube towards aspirator.
5. Resuspend in 10 mls media, gently mix, and plate in pre-labeled tissue culture plate. It is common practice to add one passage to that written on the vial.
6. On the next day, observe cells and note how well they have attached and spread. There will be some cells floating in media. Media change with 10 mls fresh media.