

EPC1/EPC2 cells: Primary human esophageal keratinocytes

EPC1/EPC2-hTERT: hTERT immortalized human esophageal keratinocytes

EPC1: Cells derived from a 65-year-old male, who underwent esophagectomy for squamous esophageal cancer following pre-operative radiotherapy and chemotherapy. Specimen was harvested from a morphologically normal site. No H&E is available.

EPC2: Cells derived from a 55-year-old male, who underwent esophagectomy for Barrett's with severe dysplasia, no cancer. No history of radiotherapy or chemotherapy. Specimen was harvested from morphologically normal proximal esophagus (upper 1/3). H&E shows normal mucosa, no inflammation, no dysplasia, and no cancer.

EPC2-hTERT: Extensive characterization has been done and published by our group.

Harada H, Nakagawa H, Oyama K, Takaoka M, Andl CD, Jacobmeier B, von Werder A, Enders GH, Opitz OG, Rustgi AK. Telomerase induces immortalization of human esophageal keratinocytes without p16^{INK4a} inactivation. *Mol Cancer Res.* 2003;1:729-38.

Andl CD, Mizushima T, Nakagawa H, Oyama K, Harada H, Chroma K, Herlyn M, Rustgi AK. Epidermal growth factor receptor mediates increased cell proliferation, migration, and aggregation in esophageal keratinocytes in vitro and in vivo. *J Biol Chem.* 2003;278:1824-30.

Takaoka M, Harada H, Deramaudt TB, Oyama K, Andl CD, Johnstone CN, Rhoades B, Enders GH, Opitz OG, Nakagawa H. Ha-Ras(G12V) induces senescence in primary and immortalized human esophageal keratinocytes with p53 dysfunction. *Oncogene.* 2004;23:6760-8.

Reagents

1. Keratinocyte-SFM (KSFM) medium supplemented with Bovine Pituitary Extract (BPE) and human recombinant Epidermal Growth Factor (EGF) (Invitrogen)...available from Cell Center stock room by a special order (#17005042 "**KERATINOCYTE SFM, COMBO**")
Make sure the catalog # as Cell Center has two different KSFM COMBOs
2. Penicillin-Streptomycin (10,000 units/ml of penicillin G sodium and 10,000 µg/ml of streptomycin sulfate)(Gibco-BRL: Cat. #15140-122)
3. Dulbecco's Phosphate-Buffered Saline (PBS)(Gibco-BRL: Cat. #14190-136)
4. Trypsin-EDTA [0.05% Trypsin, 0.53 mM EDTA-4Na](Gibco-BRL: Cat. #25300-054)
5. Soybean Trypsin Inhibitor (STI)(Sigma): 250 mg/L dissolved in DPBS (filter-steriled and stored at 4°C)(See separate protocol for STI.)
6. DMSO (Fisher Scientific)
7. Fetal calf serum (FCS)(=fetal bovine serum, FBS)

Medium: full KSFM*

KSFM: 500 ml (new bottle)

Add whole BPE vial content (25 mg) into 500 ml of KSFM (final conc. = 50 µg/ml).

Add EGF to make the final concentration 1 ng/ml (Concentrations of supplied EGF vary.

If labeled 0.02971 µg/µl which is equivalent to 30 ng/µl, add 16.7 µl (500 ng) into 500 ml of KSFM to make the final concentration of 1 ng/ml).

Add 5 ml of Penicillin-Streptomycin into 500 ml of KSFM.

*:KSFM contains 0.09 mM calcium chloride.

Freezing Medium

Add 1 ml of DMSO into 9 ml of FCS and store at -20°C .

Thaw and keep on ice prior to use.

To thaw cells

1. Thaw a cryogenic vial by incubating at 37°C in a water bath for about 2 min.
2. Transfer the content into a 15-ml conical tube containing about 10 ml of full KSFM.
3. Pellet the cells by centrifugation for 3 min at 1,000 rpm at 4°C .
4. Resuspend the cells with 15 ml of KSFM.
5. Seed the cells in a 75-cm^2 flask.

To subculture cells

1. Grow cells to 80% confluency*.
2. Add 5 ml of Trypsin-EDTA** and rock the flask gently to distribute well.
3. Remove Trypsin-EDTA by suction.
4. Incubate the dish at 37°C for 2-3 min.
5. Add ~12 ml of STI into the flask to suspend the cells***.
6. Pellet the cells by centrifugation for 3 min at 1,000 rpm at 4°C .
7. Resuspend the cells with 200-300 μl of KSFM medium, count cell number, and seed them (0.5×10^6 cells/ 75-cm^2 flask) into a new flask.

*: Subculture at 70-80% confluency. Do not allow them to grow over 100% confluency as post-confluent cells may undergo terminal differentiation.

** : No need to rinse with D-PBS prior to trypsinization

***: EPC2 cells are very sensitive to trypsin, and therefore it is very important to block trypsin activity with soybean trypsin inhibitor.

To freeze cells

1. Start from the above step #7.
2. Add pre-chilled freezing medium and mix ($\sim 1 \times 10^6$ cells/1 ml).
3. Dispense into cryogenic vials (1 ml/vial).
4. Put the cryogenic vials into isopropanol-filled freezing container. (Alternatively, wrap them with a blue-pad/paper towel and put in a closed polystyrene box).
5. Transfer the box to -80°C and keep 5 hours-overnight.
6. Transfer the cryogenic vials into a liquid nitrogen tank for long-term storage.