RWPE-2 (ATCC, Cat# CRL-11610)
Farnham Lab (150923 version)

Complete growth medium

The base medium for this cell line is provided by Invitrogen (GIBCO) as part of a kit: Keratinocyte Serum Free Medium (K-SFM), Kit Catalog Number 17005-042. This kit is supplied with each of the two additives required to grow this cell line:

1) 0.05mg/ml BPE - provided with the K-SFM kit
2) 5ng/mL EGF - provided with the K-SFM kit. NOTE: Do not filter complete medium.

To make the complete growth medium, you will need to add the following components to the base medium. Optional: Addition of penicillin (100units/mL), streptomycin (100µg/mL).

Thaw cells

1. Thaw the vial containing 1mL of RWPE-2 cells in a 37°C water bath. Transfer cells to a 10cm dish containing 9mL of growth medium.
2. Incubate in 5% CO^2 incubator at 37°C overnight.
3. Discard medium carefully, add 10mL of fresh growth medium.

Subculturing

For cell growth in 15cm dish:

1. Grow cells until about 80% (+/-10%) confluence.
2. Remove and discard culture medium.
3. Wash cells carefully with warm PBS.
4. Trypsinize cells with 6-8ml of 0.05% trypsin and 0.53mM EDTA at 37^{o}C for 5-8min.
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach.
5. Neutralize trypsin with 12-14ml of PBS+2%FBS.
6. Transfer cell suspension to centrifuge tube and spin down cells at 125rcf for 5min at room temperature.
7. Discard supernatant, re-suspend cells with growth medium (K-SFM) and passage at 1:3 to 1:5 ratios (An inoculum of 2 X 10^4 to 4 X 10^4viable cells/cm^2 is recommended).
8. Incubate cultures at 37°C. Renew medium every 2 days. We recommend that you maintain cultures at a cell concentration between 4 X 10^4 and 7 X 10^4 cells/cm^2.

Cells grown under serum-free or reduced serum conditions may not attach strongly during the 24 hours after subculture and should be disturbed as little as possible during that period.

Freeze medium: Complete growth medium supplemented with 10% (v/v) DMSO.