Product description

Immortalized neonatal foreskin keratinocyte line useful for researchers studying inflammation and pathogenesis.

Name: TERT HFK clone 398

Alternate name: N/A

Organism: Human

Tissue: Foreskin

Growth properties: Adherent

Model: Immortalized cell line

CRISPR Edited: No

Conditional: No

Production details: Keratinocyte cell line immortalized using TERT.

Biosafety level: 1

Contributor(s)

Inventor: Aloysius Klingelhutz
Institute: The University of Iowa

Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapour, until ready for use.

Recommended medium: Keratinocyte Serum Free Medium (K-SFM) (Gibco™, 17005042) supplemented with 0.16 ng/mL EGF and 25 ug/mL BPE. Do not keep media for more than 1 month past adding supplements. Do not freeze and thaw media.

Change media every other day. The cell line 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.

Subculture: Split sub-confluent cultures (70-90%) 1:4 every 4 to 5 days using 0.05% trypsin/EDTA; 5% CO2; 37°C;5-10 min. Tap plate and view under scope to see if cells have lifted. Add 4 ml PBS-A containing 2% FBS to inactivate the trypsin and centrifuge at 1200 rpm for 5 minutes to wash.

Recommended cryopreservation medium: K-SFM with 10% DMSO and 15% FBS.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

1. Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.
2. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining (this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.
3. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
4. Transfer the remaining cell suspension to a 15 mL conical tube containing 5 mL PBS using a pipette.
5. Rinse the vial with 1 mL of PBS and add it dropwise to the cells, while gently swirling the tube.
7. Centrifuge the cell suspension at **1200 rpm for 5 minutes** at room temperature.
9. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend in 10 mL fresh medium, mix gently and plate in an appropriate culture flask.
10. Examine the cultures after 24 hours. Some cell death will be observed and that is normal. Change medium and subculture following instructions above.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: TERT HFK clone 398 was invented by Aloysius Klingelhutz (CancerTools.org #154458).