# Ker-CT

## CRL-4048™

### Description

Ker-CT is an hTERT-immortalized keratinocyte cell that was isolated from the foreskin of a male patient. This cell line was deposited by J Shay and can be used in toxicology research.

**Organism:** *Homo sapiens*, human
**Cell Type:** keratinocyte
**Tissue:** Skin; Foreskin
**Age:** neonate
**Gender:** Male
**Morphology:** epithelial
**Growth properties:** Adherent
**Disease:** Normal

### Storage Conditions

**Product format:** Frozen

### Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

### BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to

understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

Cells contain SV40 sequences

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

## Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

## Growth Conditions

**Temperature:** 37°C
**Atmosphere:** 95% Air, 5% $CO_2$

## Handling Procedures

**Unpacking and storage instructions:**

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 vapor, until ready for use.

**Complete medium:** KGM-Gold™ BulletKit™(Lonza 00192060). Note: Discard the GA-1000

**Handling Procedure:** To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt.

If storage of the frozen culture is necessary upon arrival, store the vial in liquid nitrogen vapor phase and NOT

at –70°C. Storage at –70°C will result in loss of viability.

1. Prepare a 25 $cm^2$ or a 75 $cm^2$ culture flask containing the recommended complete culture medium. Prior to the addition of the vial contents, the vessel containing the growth medium should be placed in the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6) and to avoid excessive alkalinity of the medium during recovery of the cells.
2. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All operations from this point on should be carried out under strict aseptic conditions.
4. Thaw the frozen vial at 37°C and resuspend the cells in 5mL of complete growth medium. Count the cells and seed at recommended seeding densities. DO NOT centrifuge after thawing to remove DMSO.
5. Incubate the culture at 37°C in a suitable incubator.
6. Change to fresh medium after the cells attached, usually 6-12 hours later, to remove DMSO and FBS.

**Subculturing procedure:** Volumes used in this protocol are for 75 $cm^2$ flasks; proportionally reduce or increase amount of dissociation solutions for culture vessels of other sizes.

1. Remove and discard spent medium.
2. Briefly rinse with HBSS (ATCC 30-2213), 1 mL/25 $cm^2$ and discard rinse solution.
3. Add trypsin for primary cells (ATCC PCS-999-003), 1mL / 25$cm^2$. Place at 37oC for 4-6 minutes, until 90% of the cells have detached.
4. Rap flask gently to ensure cells are detached. Add 2% FBS in D-PBS, 1 mL/25$cm^2$ to neutralize trypsin.
5. Centrifuge cells at 250 x g for 5 min at room temperature.

6. Remove supernatant. Resuspend pellet in 6.0 to 8.0 mL Complete Growth Medium.
7. Count cells, and seed 5.0 x $10^3$ to 8.0 x $10^3$ viable cells/$cm^2$ to new culture vessels.

**Medium Renewal:** Every 2-3 days.

As the cells become more confluent, increase the volume of media as follows: under 25% confluence feed cells 5 mL per 25 $cm^2$, 25-45% confluence then feed cells 7.5 mL per 25 $cm^2$, over 45% confluence then feed cells 10 mL per 25 $cm^2$.

**Reagents for cryopreservation:** Fetal bovine serum supplemented with 10% (v/v) DMSO (ATCC 4-X)

## Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Ker-CT (ATCC CRL-4048)

## References

References and other information relating to this material are available at www.atcc.org.

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