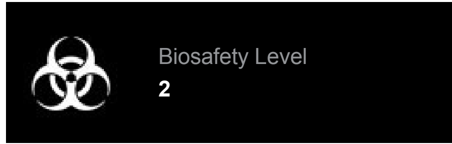
Product Sheet

Ker-CT (ATCC® CRL-4048™)

Please read this FIRST



Intended Use

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

Complete Growth Medium

KGM-Gold™ BulletKit™(Lonza 00192060)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: Ker-CT (ATCC® CRL-4048™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Immortalization Method: human telomerase and CDK4 using retroviral pBABE-puro hTERT and pSRalphaMSU expressing mouse CDK4
Tissue: foreskin
Disease: normal
Cell Type: keratinocyte
Age: neonatal
Gender: male
Morphology: epithelial
Growth Properties: adherent
DNA Profile: D5S818: 12
D13S317: 11, 13
D7S820: 10, 11
D16S539: 9, 11
vWA: 15, 16
Amelogenin:
TPOX: 8
CSF1PO: 7, 11
TH01: 6, 9
Cytogenetic Analysis: Cytogenetic analysis was performed on G-banded metaphase cells from the human cell line Ker-CT and two abnormal male clones were detected. Clone 1 demonstrated trisomy 5 and trisomy 20. Clone 2 demonstrated trisomy 5 and four copies of chromosome 20.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

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.

Handling Procedure for Flask Cultures

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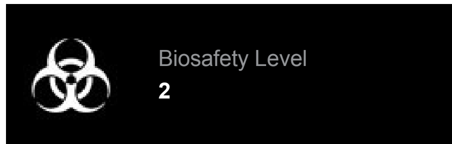

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The flask was seeded with cells, incubated, and completely filled with medium at ATCC to prevent loss of cells during shipping.Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.

1. Using an inverted microscope (preferably equipped with phase contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C with 5% CO2 until they are ready to be subcultured.
3. **If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to 25 cm^2 flask. Incubate at 37°C with 5% CO_2 until cells are ready to be subcultured.

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 / 25cm^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/25cm^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.

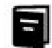
Medium Renewal: Every 2-3 days.
As the cells become more confluent, increase the volume of media as follows: under 25% confluence feed cells 5 mL per 25 cm^2, 25-45% confluence then feed cells 7.5 mL per 25 cm^2, over 45% confluence then feed cells 10 mL per 25 cm^2.

Cryopreservation Medium

90% FBS, 10%DMSO

Comments

The Ker-CT cell line is positive for telomerase, failed to senesce, and was proliferating after more than 250 population doublings Ref



References

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

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

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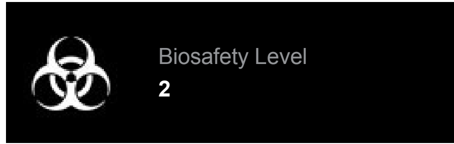

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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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