CW1474

CRL-3529™

Description

CW1474 is a cell line derived from an esophageal adenocarcinoma isolated from a human donor.
Organism: *Homo sapiens*, human
Tissue: Esophagus
Gender: Male
Morphology: epithelial-like
Growth properties: Adherent
Disease: Adenocarcinoma
Cells per vial: Approximately 2.0 to 3.0 x 10^6

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

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 1

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.

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

Complete medium:

The base medium for this cell line is L-WRN Conditioned Medium.

To make the complete medium, Supplement 245 mL of L-WRN Conditioned Medium

with the following additives:

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Note: Refer to the product sheet for CRL-3279 for Wnt-3A, R-spondin and Noggin Conditioned Medium protocol.

- **25 mM SB202190: use 100 L/245 mL L-WRN Conditioned Medium**

  To prepare 25 mM SB202190 solution, combine:

- 1 vial of 830 µg SB202190 (ATCC ACS-7211)
- 100 µL **DMSO** (ATCC 4-X)
- **500 nM A83-01: use 100 L/245 mL L-WRN Conditioned Medium**

  To prepare 500 nM A83-01 solution, combine:

- 1 vial of 53 µg A83-01 (ATCC ACS-7209)
- 100 µL **DMSO** (ATCC 4-X)
- **10 nM Gastrin: use 473 L/245 mL L-WRN Conditioned Medium**

  To prepare 10 nM Gastrin solution, combine:

- 1 vial of 5.5 µg Gastrin (ATCC ACS-7208)
- 500 µL of L-WRN Conditioned Medium
- **200 ng/mL FGF-10: use 2 mL/245mL of L-WRN Conditioned Medium**

  To prepare 200 ng/mL FGF-10 solution, combine:

- 2 vials of 25 µg FGF-10 (ATCC ACS-7204)
- 2 mL of L-WRN Conditioned Medium
- **10 mM Nicotinamide: use 2.5 mL / 245mL L-WRN Conditioned Medium**

  To prepare 10 mM Nicotinamide solution, combine:

- 1 vial of 310 mg Nicotinamide (ATCC ACS-7214)
- 2.5 mL of L-WRN Conditioned Medium

  Note: The complete culture medium with additives above should be protected from light and it is stable for 14 days when store at 2 to 8°C. Due to the limited stability, following additive will be spiked separately.

  ROCK Inhibitor Supplementation:`

- **10 M ROCK Inhibitor Y27632: use 1 L 10 mM stock ROCK inhibitor/ 1 mL of complete culture medium**

  To prepare 10 mM of ROCK Inhibitor Y-27632 stock solution, combine:

- 10 mg ROCK Inhibitor Y27632 (ATCC ACS-3030)
- 3.1 mL molecular grade water (ATCC 60-2450)

  Pipette to mix, store in working aliquots at -20°C. The solution is stable for 6 months when prepared and stored as directed. Do not repeat freeze/thaw.

Note: Only supplement 10 µM ROCK Inhibitor Y27632 into desired volume of complete culture medium when performing either fluid changes or subculture.

Handling Procedure:

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70° C. Storage at -70°C will result in loss of viability.

1. 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).
2. 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 of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 x *g* for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6). pH (7.0 to 7.6).
5. Incubate the culture at 37° C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of TrypLE™ Express Enzyme (1X), no phenol red (ThermoFisher catalog#: 12604-013) to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium(without rock inhibitor) and aspirate cells by gently pipetting.
5. Centrifuge cells at 150 to 400 xg for 8 to 12 minutes to remove dissociation agent.
6. Add appropriate aliquots of the cell suspension to new culture vessels. Cultures can be established between 4.0 x 10^4 and 7.0 x 10^4 viable cells/cm^2.
7. Incubate cultures at
   Atmosphere: air, 95%; carbon dioxide (CO_2), 5%
   Temperature: 37°C
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Interval: Subculture at 50-80% confluence. Maintain cultures at a cell concentration between 4.0 X 10^4 and 7.0 X 10^4 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:3 is recommended
Medium Renewal: 2 to 3 times per week

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: CW1474 (ATCC CRL-3529)

References

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

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