ISE6

CRL-3576™

Description

ISE6 is a tick-derived cell line. This product is an ATCC manufactured and accessioned progeny of CRL-11974 cited in US Pat. No. US 5,869,335.
Organism: *Ixodes scapularis*
Tissue: Embryo
Age: embryo
Morphology: Epithelial-like and/or round; neuronal-like
Growth properties: Adherent
Cells per vial: 1.0 x 10^7

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: 30-34°C
Atmosphere: 100% Air

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:

The complete medium for this cell line is L15B (see media formulation supplemental data) + 5% heat-inactivated FBS (ATCC 30-2020) + 0.1% Lipoprotein-cholesterol concentrate

ATCC Medium: L-15B

Stock A

- CoCl2.5H2O (Sigma, C8661-25G) ……..….. 2 mg
- CuSO4.H2O (Sigma C8027-500G) …… 2 mg
- MnSO4.H2O (Sigma M7899-500G) …… 16 mg
- ZnSO4.7H2O (Sigma Z0251-100G) …… 20 mg
- Cell culture grade water (Invitrogen 15230162) …… 10 mL

Agitate well at a low speed until the solution becomes clear. After adding 100 µL stock A to Stock D, store in -20°C.

Stock B

- Na2MoO4.2H2O (Sigma M1651-100G) …… 2 mg
- Cell culture grade water (Invitrogen 15230162) …… 10 mL

Agitate well at a low speed until the solution becomes clear.

After adding 100 µL stock B to Stock D, store in -20°C.

Stock C

- Na2SeO3 (Sigma S5261-10G) …… 2 mg
- Cell culture grade water (Invitrogen 15230162) …… 10 mL

Agitate well at a low speed until the solution becomes clear. After adding 100 µL stock C to Stock D, store in -20°C.

Stock D

- Ascorbic acid (Sigma A4403-100MG) …….. 100 mg
- Glutathione, reduced (Sigma G6013-5G) ……100 mg
- FeSO4.7H2O (Sigma F8633-250G) ……... 5 mg
- Cell culture grade water (Invitrogen 15230162) …… 9.7 mL
- Stock A …….. 100 µL
- Stock B …….. 100 µL

- Stock C …….. 100 µL

Add components listed above in order. For example, after 100 mg Ascorbic acid is dissolved, add 100 mg Glutathione. Add 5mg FeSO4.7H2O after Glutathione is dissolved. Once all of these components are dissolved well, combine this solution and 100 µL Stock A, 100 µL Stock B, 100 µL Stock C lastly to make 10 mL Stock D. After adding 1 mL Stock D into 1 L of L-15B, store at -20°C.

* Note: FeSO4.7H2O is light sensitive and rapidly oxidized by heat.

Stock E

- p-aminobenzoic acid (Sigma A9878) ……..…….. 10 mg
- Cyanocobalamine (B12) (Sigma V6629-250MG) ……... 5 mg
- d-Biotin (Sigma B4639-100MG) ……..1 mg
- Cell culture grade water (Invitrogen 15230162) ……..…… 10 mL

Agitate well at a low speed until the solution becomes clear. After adding 1 mL Stock E into 1 L of L-15B, store in -20°C.

Final product L-15B

- L-15 powder (Gibco 41300039) …… 1 pkg (1 package)
- L-aspartic acid (Sigma A7219-100G) …….. 0.299 g
- L-glutamine (Sigma G8540-25G) ……..0.292 g
- L-proline (Sigma P5607-25G…… 0.300 g
- L-glutamic acid (Sigma G8415-100G) …… 0.490 g
- a-ketoglutaric acid (Sigma K1128-5G) …… 0.299 g
- D-glucose (Sigma G7021-100G) ……..14.41 g
- Stock D …….. 1.0 mL
- Stock E …….. 1.0 mL
- Cell culture grade water (Invitrogen 15230162) ……..……700 mL (Final 1000 mL)

*Note: Use fresh L-glutamic acid: expired in 1 year at 4 °C

*Note: Stir slowly until all components are dissolved well without heating (< 1 hr). While stirring, protect the media from light.

Adjust pH to 7.2 by adding 10N NaOH (~0.5 mL).

Bring the volume up to 1L with Cell culture grade water.

Filter the media with 0.22 µm aPES filter unit. Aliquot 500 mL and store at 4 °C. Media expires 6 months from preparation date or the expiration date of the shortest shelf life component.

Handling Procedure:

Handling Procedure for Frozen Cells

To insure 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.

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.

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 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. 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).

5. Incubate the culture at 31°C in a suitable incubator. A free gas exchange with

atmospheric air is recommended if using the medium described on this product.

Subculturing procedure:
Volumes used in this protocol are for non-vented(plug cap) 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® Plug seal cap T-75 flasks (catalog #430720U) are recommended for subculturing this product.

1. Do not use trypsin to detach cells. At the time of subculture, the medium in the culture flask should first be replaced with fresh culture medium.
2. Tapping the flask may work if cells are loosely attached. The cells are then flushed off the growth surface using a serological pipette to direct a stream of medium at the cell layer until all have been detached.
3. Once collected, detached cells can be counted and seed into a new flask at appropriate seeding density without centrifugation.
   It is recommended to subculture once a week. Do not change media before 7 days. If cells are not ready for subculture at Day 7, media change should be performed. If cells appear to be detached the day following the media change, collect and centrifuge the floating cells and proceed with subculture as previously described. Combine cell pellet from the floating cells with the collected adherent cells before performing a cell count.
4. Add appropriate aliquots of the cell suspension to new culture vessels.
   Cultures can be established between 2.0 x 10^{54} and 4.06.0 x 10^5 viable cells/cm^2.
5. Incubate cultures at 32.0°C.

Interval: Maintain cultures at a cell concentration between 1.0 X 10^{54} and 5.0 X 10^5 cell/cm^2.
Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:68 is recommended
Medium Renewal: Once per week
Reagents for cryopreservation: Complete culture medium + 5% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: ISE6 (ATCC CRL-3576)

References

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

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Revision

This information on this document was last updated on 2025-10-05

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