Tick Cell Line ISE6 Derived from *Ixodes scapularis* Embryos

Catalog No. NR-12234

For research use only. Not for use in humans.

Contributor:
Ulrike G. Munderloh, Professor, Department of Entomology, University of Minnesota, St. Paul, Minnesota, USA

Manufacturer:
BEI Resources

Product Description:
The cell line, ISE6, was derived using whole embryos from black-legged ticks *(Ixodes scapularis)*. The primary culture was obtained from embryos at an age of 25 days postonset of oviposition. Cell line ISE6 was established by subculturing the primary culture using techniques described in the literature.[1,2,3]

Material Provided:
Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (70%), fetal bovine serum (20%) and DMSO (10%) cryopreservative. Sufficient cells are provided to initiate at least one new culture. The cell count, expressed as cells per vial, is shown on individual certificates of analysis for each lot.

Packaging/Storage:
This product was packaged aseptically in cryovials. It should be stored at -100°C or colder, preferably in the vapor phase of a liquid nitrogen freezer. Storage at -70°C will result in loss of viability. To ensure the highest level of viability, the vial should be thawed and the culture initiated as soon as possible upon receipt. Any warming of the product during shipping and transfer must be avoided, as this will adversely affect the viability of the product after thawing. For transfer between freezers and shipping, the cells may be placed on dry ice for brief periods, although use of a portable liquid nitrogen carrier is preferred. Please read the following recommendations prior to reconstituting this material.

Safety Precautions:
When handling frozen vials, it is highly recommended that protective gloves, lab coat and full-face mask be worn. Even brief exposure to the ultra-cold temperature can cause tissue damage from frostbite. Also, some vials may slowly fill with liquid nitrogen if they have been immersed during cryogenic storage. When thawing, the liquid nitrogen may rapidly expand as it changes to gas, breaking the vial or cap with explosive force, sending debris flying with enough velocity to cause injury. Store and use in areas with adequate ventilation.

Thawing and Growth:
Prior to thawing the tick embryo cells, prepare growth medium (GM) for use. Tick embryo cells are grown in L-15B medium (Appendix I) containing 5% fetal bovine serum (ATCC® 30-2020™), 5% tryptose phosphate broth (BD Bacto™ 260300) and 0.1% of bovine lipoprotein cholesterol concentrate (MP Biomedicals, LLC 191476).[1,4] This GM is formulated for use with ambient atmosphere at 30 to 34°C.

Rapidly thaw the vial of tick embryo cells in a 37°C water bath with gentle agitation. To reduce the risk of contamination, keep the cap and O-ring of the vial out of the water and repeatedly check the cap for tightness during thawing. Remove from the water bath immediately when thawed. Dry the vial with a sterile wiper, decontaminate using a wiper soaked with 70% isopropyl alcohol, and let the vial air dry. Aseptically open the vial, remove the vial contents and add to 5 mL of GM in a 25 cm^2 tissue culture flask. Incubate the culture at 30 to 34°C overnight. Gently replace the GM with fresh pre-warmed GM the next day and incubate the culture at 30 to 34°C until the cell sheet is approximately 80% confluent.

Sub-culture procedure. Aseptically remove the GM from the tissue culture flask and replace it with fresh pre-warmed GM. Gently flush off the cells from the flask surface using a pipette. *Note: Enzymatic treatment to separate the cells is not recommended as it may damage the cells.* Perform a cell count and add appropriate aliquots of the cell suspension to new culture vessels at a sub-cultivation ratio of 1:5 to 1:10. Adjust the volume of GM to 15 to 20 mL for a 75 cm^2 flask. Incubate cultures at 30 to 34°C until the cell sheet is approximately 80% confluent.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: Tick Cell Line ISE6 Derived from *Ixodes scapularis* Embryos, NR-12234."

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:
You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S.

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

NR-12234 is claimed in U.S. Patent Numbers 5,869,335 and 5,928,879, and the continuations, continuations-in-part, re-issues and foreign counterparts thereof.[1]

References:

1. Munderloh, U. G., Personal Communication.
2. Munderloh, U. G., et al. "Establishment, Maintenance and Description of Cell Lines from the Tick *Ixodes scapularis*." J. Parasitol. 80 (1994): 533-543. PubMed: 8064520.
3. Obonyo, M., et al. "*Borrelia burgdorferi* in Tick Cell Culture Modulates Expression of Outer Surface Proteins A and C in Response to Temperature." J. Clin. Microbiol. 37 (1999): 2137-2141. PubMed: 10364575.
4. Munderloh, U. G. and T. J. Kurtti. "Formulation of Medium for Tick Cell Culture." Exp. Appl. Acarol. 7 (1989): 219-229. PubMed: 2766897.

ATCC® is a trademark of the American Type Culture Collection.

Support Provided by NIAID

BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

APPENDIX I: TICK CELL L-15B GROWTH MEDIUM

L-15B Medium:	
Cell culture grade water	1000 mL
L-15 Powder (Sigma L4386)	13.8 g
L-Aspartic acid	0.449 g
L-Glutamine	0.5 g
L-Proline	0.45 g
L-Glutamic acid (less than a year old)	0.49 g
α-Ketoglutaric acid	0.449 g
D-Glucose	18.01 g
Mineral Stock Solution D (see below)	1 mL
Vitamin Stock (see below)	1 mL
10 N NaOH	0.75 mL

Mineral Stock Solution D:	
Ascorbic acid	1000 mg
Reduced Glutathione	1000 mg
$FeSO_4$•7H2O	50 mg
Stock Solution A (see below)	1 mL
Stock Solution B (see below)	1 mL
Distilled water	100 mL

Stock Solution A:	
$CoCl_2$•$6H_2O$	20 mg
$CuSO_4$•$5H_2O$	20 mg
$MnSO_4$•H_2O	160 mg
$ZnSO_4$•$7H_2O$	200 mg
Distilled water	100 mL

Stock Solution B:	
$NaMoO_4$•$2H_2O$	20 mg
Na_2SeO_3	20 mg
Distilled water	100 mL

Vitamin Stock Solution:	
p-Aminobenzoic acid	100 mg
Vitamin B12	50 mg
Biotin	10 mg
Distilled water	100 mL

Reconstitute L-15B growth media in 500 to 700 mL cell culture grade water in a volumetric flask that has been high heat sterilized (180°C or higher for at least 2 hours) to remove endotoxins. Stir in all remaining components until incorporated and then, using cell culture grade water, bring the final volume up to 1 L. Wrap the flask in aluminum foil to protect from light and stir slowly for another 1 to 1.5 hours. Upon completion, filter the L-15B media through a 0.22 µm filter into storage bottles. The media should be between pH 5.5 and 6.5 and stored at 4°C protected from light.