

**Tick Cell Line ISE6 Derived from *Ixodes scapularis* Embryos**

**Catalog No. NR-12234**

**For research use only. Not for use in humans.**

**Contributor:**

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**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 post onset of oviposition. Cell line ISE6 was established by subculturing the primary culture using techniques described in the literature.<sup>1,2,3</sup>

**Material Provided:**

Each vial contains approximately 1 mL of cell culture suspension frozen in cell growth medium (80%), fetal bovine serum (10%) 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 screw-capped plastic 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).<sup>1,4</sup> 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<sup>2</sup> 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<sup>2</sup> 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 \(BMBL\)](#), 6th ed. Washington, DC: U.S. Government Printing Office, 2020.

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**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.

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**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 <sub>4</sub> •7H <sub>2</sub> O	50 mg
Stock Solution A (see below)	1 mL
Stock Solution B (see below)	1 mL
Distilled water	100 mL

*Stock Solution A:*

CoCl <sub>2</sub> •6H <sub>2</sub> O	20 mg
CuSO <sub>4</sub> •5H <sub>2</sub> O	20 mg
MnSO <sub>4</sub> •H <sub>2</sub> O	160 mg
ZnSO <sub>4</sub> •7H <sub>2</sub> O	200 mg
Distilled water	100 mL

*Stock Solution B:*

NaMoO <sub>4</sub> •2H <sub>2</sub> O	20 mg
Na <sub>2</sub> SeO <sub>3</sub>	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.