ATCC

Product Sheet

L10BIOBR-MAPKK (ATCC® CRL-2771™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
2

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

Ham's F10 medium supplemented with 50 ng/ml TPA (Sigma Catalogue No. P-8139) and 7% horse serum

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: L10BIOBR-MAPKK (ATCC® CRL-2771™)

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: *Mus musculus*, mouse
Strain: B10.BR
Cell Type: melanocyte
Age: newborn
Morphology: melanocyte
Growth Properties: adherent

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

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.
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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 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 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Subculturing Procedure

Protocol:
1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution 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 and aspirate cells by gently pipetting.

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5. Add appropriate aliquots of the cell suspension to new culture vessels.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/sq. cm. is recommended.
6. Incubate cultures at 37°C.

Interval: Subculture when cells reach a concentration of 4 X 10(4) cells/sq. cm.
Subcultivation Ratio: A subcultivation of 1:6 to 1:8 is recommended
Medium Renewal: Two to three times weekly

Cryopreservation Medium

Cryoprotectant Medium
Complete growth medium supplemented with 5% (v/v) DMSO.Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

Comments

The L10BIOBR-MAPKK cell line (ATCC CRL-2771) was derived by infecting the immortalized murine melanocyte cell line, L10BIOBR, with pBABE which encodes a constitutively active MAPKK. The vector contains the SV40 viral DNA sequences and the puromycin resistance gene. The cells were selected in medium containing puromycin. The introduction of the MAPKK gene into melanocytes leads to tumorigenesis in nude mice, activation of the angiogenic switch and increased production of the proangiogenic factor, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs). Activation of MAP kinase signaling may be an important pathway involved in melanoma transformation. Inhibition of MAP kinase signaling may be useful in the prevention and treatment of melanoma. The L10BIOBR-MAPKK cell line and the corresponding negative control, L10BIOBR-GFP (CRL-2770), are a model for melanoma tumorigenesis and signal transduction [PubMed: 12514183].

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

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Additional information on this culture is available on the ATCC web site at www.atcc.org.
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