ATCC

Product Sheet

TBM-54 (ATCC® CRL-2051™)

Please read this FIRST

Biosafety Level
1

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

A 7:3 mixture of Coon's F-12 and Leibovitz's L-15 medium modified to contain 103 mM NaCl and 25 mM NaHCO3 with 1 nM dexamethasone, 30 nM spermine, 250 nM spermidine, 130 nM putrescine, 0.01 mg/ml insulin, 95%; heat-inactivated fetal bovine serum, 5%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: TBM-54 (ATCC® CRL-2051™)

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: *Bufo marinus*, toad, tropical
Tissue: urinary bladder
Cell Type: Epithelial
Morphology: epithelial
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

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 28°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath. All of the operations from this point on should be carried out under strict aseptic conditions.

- Transfer the cell suspension and dilute it with the recommended culture medium in a 25 cm. sq. culture flask (see specific batch information above for dilution ratio); incubate at 28°C with 5% CO_2 in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the vial contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO_2 will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL
Three times weekly.

SUBCULTURE PROCEDURE
Remove medium, add fresh trypsin (0.25%) - EDTA (0.03%) solution, rinse quickly and remove. Allow flasks to remain at room temperature until cells start to detach. Add fresh medium, aspirate and dispense into new culture flasks. Subcultivation ratio: 1:3 to 1:8 is recommended.

Handling Procedure for Flask Cultures

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HANDLING PROCEDURE FOR FLASK CULTURES (MONOLAYER)
The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Remove all of the medium (which can be saved and used as fresh medium) except for a sufficient volume (5-10 ml) to cover the floor of the flask. Incubate at 28°C in a 5% CO_2 in air atmosphere. Sometimes in transit the cultures are handled roughly and most of the cells become detached and float in the culture medium. If this has occurred remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Draw off the excess supernatant medium, resuspend the cells in 10 ml of the culture medium and plant the entire cell suspension in a single flask of suitable size (about 25 sq. cm.).

Subculturing Procedure

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended
Medium Renewal: 3 times per week
Remove medium, and rinse the monolayer with fresh 0.25% trypsin solution. Remove the trypsin solution, and let the culture sit at room temperature until the cells detach (about 5 to 10 minutes). Add fresh medium, aspirate and dispense into new flasks.

Comments

TBM-54 is an epithelial cell line derived in 1977 from the urinary bladder of a tropical toad collected in the Dominican Republic.
The cells resemble the granular cell type of the urinary bladder.
They have dense granules near the apical surface characteristic of the granular cell type found in the intact bladder.
These cells form an epithelium with typical tight junctions and gap junctions and they form multilayers with one layer of undifferentiated cells interposed between the apical layer and the supporting structure.
They exhibit hormone sensitive transepithelial electrical resistance, actively transport sodium and are subject to hormonal control in culture.
Development of transepithelial electrical resistance (R) and transport rate (Isc) are dependent on time and the density of cells seeded, but steady state Isc and R is characteristic of the cell line and independent of seeding density.
TBM-54 cells do not respond to vasopressin.
Analogues of cAMP increase sodium transport and urea permeability but do not effect water permeability.
Aldosterone increases sodium transport in a manner similar to that observed in intact bladder.
TBM-54 cells exhibit an excellent response to mineralocortioids in serum free medium.

References

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

Biosafety Level: 1

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

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. 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.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet,

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