TBM-54

CRL-2051™

Description

Organism: *Bufo marinus*, toad, tropical
Cell Type: epithelial cell
Tissue: Urinary bladder
Morphology: epithelial
Growth properties: Adherent

Storage Conditions

Product format: Frozen

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.

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: 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%

Handling Procedure: 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.
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.
Reagents for cryopreservation: Complete growth medium supplemented with 5% (v/v) DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: TBM-54 (ATCC CRL-2051)

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 2024-10-25

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