VMM17

CRL-3228™

Description

Organism: *Homo sapiens*, human
Cell Type: melanocyte
Age: 64 years
Gender: Female
Morphology: Epithelial-like
Growth properties: Adherent
Disease: Melanoma; Stage IIIB

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

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.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

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: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, ATCC 30-2001. To make the complete growth medium, add the following

components to the base medium: fetal bovine serum (ATCC 30-2020) to a final concentration of 10%.
Handling Procedure: 1 amp --> 1 T-75

Thaw ampoule in 37ºC water bath for approximately 2 minutes. Transfer thawed cell suspension to a 15.0 mL centrifuge tube containing 9 mL complete medium. Mix suspension by gentle inversion. Remove 0.5-1.0 mL for cell count. Centrifuge remaining suspension in the 15mL centrifuge tube at 175-195 x g (1000 rpm in an IEC HN SII centrifuge or equivalent) for 5 minutes, RT°. Discard supernatant and gently resuspended pellet in 5ml fresh complete medium. Transfer 5 ml re-suspended cells into 1 T-75 flask containing 10 ml fresh medium. Place the cells in a 5% CO_2 incubator @ 37ºC

Subculturing procedure:

Volumes are for a T-75 flask; Adjust accordingly

1. Remove and discard the cell culture medium from the flask.
2. Rinse the cell monolayer with Dulbecco's PBS without calcium or magnesium and remove.
3. Add 3 to 4 ml of the trypsin-EDTA solution, rotate flask to rinse cell monolayer, remove trypsin solution, and incubate at 37ºC.
4. Once the cells appear to be detached, add 10 ml of complete growth medium with a pipette to the cell suspension to inactivate the trypsin. Gently wash any remaining cells from the growth surface of the flask. Check the cells with the microscope to be sure that most (>95%) are single cells. If cell clusters are apparent, continue to disperse the cells with gentle pipetting.
5. Subculture as necessary.
6. Place the flask back into the incubator. Examine the culture the following day to ensure the cells have reattached and are actively growing.
7. Repeat when cells reach confluence.

Culture maintenance: Cultures are grown @ 37°C in a 95% air, 5% CO_2 environment. Medium change every 2-4 days.
Reagents for cryopreservation: Fetal bovine serum supplemented with 10% (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: VMM17 (ATCC CRL-3228)

References

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

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