

## **CRL-3222**<sup>™</sup>

## **Description**

Organism: Homo sapiens, human

Tissue: Skin Age: 54 years Gender: Female

Morphology: epithelial

**Growth properties:** Adherent

**Disease:** Melanoma **Volume:** 1.0 mL

## **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<sub>1</sub>

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



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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<sub>2</sub>

## 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 Dulbecco's

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Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

**Subculturing procedure:** Volumes are given for a 75 cm2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

- 1. Remove and discard culture medium.
- 2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) (ATCC 30-2200) or 0.05% (w/v) Trypsin-0.02% EDTA solution (ATCC PCS-999-003) 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.
- 5. Add appropriate aliquots of the cell suspension to new culture vessels.
- 6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:8 is recommended Medium Renewal: Every 2 to 3 days

**Reagents for cryopreservation:** Complete growth medium 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: A375-MA1 (ATCC CRL-3222)

### References

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

# **A375-MA1** CRL-3222

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#### Revision

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