ICM-BROD-0047-C71
DM-23™

Description

- **Organism** Homo sapiens, human
- **Tissue** Brain
- **Morphology** neuronal
- **Growth properties** Adherent
- **Disease** Glioblastoma; Recurrent
- **Cells per vial** $\geq 1.0 \times 10^6$
- **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 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% CO₂

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**

  Renaissance Essential Tumor Medium™ basal media and supplement (Cellaria Bio cat# CM-0001) supplemented with 5% Fetal Bovine Serum (FBS; ATCC 30-2020) and 25 ng/mL Cholera Toxin (Sigma Aldrich cat# C8052).

  Prepare media according to the manufacturer’s instructions.

- **Handling Procedure**

  To ensure 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.

  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 200 x g for 5 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 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% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

- **Subculturing procedure**

  1. Passage cells when the culture has reached approximately 70% to 80% confluence.
  2. Warm TrypLE (Thermofisher cat# 12605010) and complete growth media to room temperature.
  3. For each flask, carefully aspirate the spent media without disturbing the monolayer.
  4. Rinse the cell layer one time with 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual medium.
  5. Add room temperature TrypLE (1 to 2 mL for every 25 cm²) to each flask.
  6. Gently rock each flask to ensure complete coverage of the TrypLE solution over the cells, and then aspirate the excess fluid off of the monolayer.
  7. Observe the cells under the microscope.
  8. When the majority of cells appear to have detached (typically 2-5 minutes), quickly add an equal volume of the complete growth medium to each flask.
  9. Transfer the dissociated cells to a sterile centrifuge tube.
  10. Centrifuge the cells at 200 x g for 5 minutes.
  11. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
  12. Count the cells and seed new culture flasks at a density of 3 to 5 x 10⁴ viable cells per cm². Prior to seeding, aspirate the coating and discard the coating laminin solution from the vessel.
  13. Place newly seeded flasks in a 37°C, 5% CO₂ incubator for at least 24 to 48 hours before processing the cells further.
  14. Perform a complete medium change every 3-4 days or as needed.

- **Reagents for cryopreservation** Complete growth media containing 10% DMSO (ATCC 4-X)

  Note: Lots manufactured by ATCC after May 2020 do not have cholera toxin in cryopreservation medium.

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**Material Citation**

www.atcc.org
If use of this material results in a scientific publication, please cite the material in the following manner: HCM-BROD-0047-C71 (ATCC PDM-23)

References

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

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Revision

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