HCM-BROD-0761-C71

PDM-499™

Description

A patient-derived next-generation cancer model generated by the Human Cancer Models Initiative (HCMI). HCM-BROD-0761-C71 (ATCC No. PDM-499) was isolated from a recurrent glioblastoma from brain tissue. This tumor-derived model can be used in basic research and pharmacological screening applications. Please check the GDC for the availability of any data for this model.

Organism: *Homo sapiens*, human
Tissue: Brain
Morphology:

neuronal

Growth properties: 2D adherent
Disease: Glioblastoma; Primary
Cells per vial: ≥ 1.0 x 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% CO_2

Handling Procedures

Complete medium:

NeuroCult NS-A Basal Medium (StemCell Technologies #05750) with NS-A Proliferation Supplement (StemCell Technologies #05754) + 20 ng/mL EGF (StemCell Technologies #78003.1) + 20 ng/mL bFGF (Peprotech #AF-100-15) + 2 µg/mL Heparin (StemCell Technologies #07980).

Prepare media according to the manufacturer's instructions: Stem Cell Technologies Catalog #5751

Handling Procedure:

Culture vessels must be pre-coated with laminin prior to seeding with cells.

Coating procedure (perform all steps in a biosafety cabinet)

1. Thaw (Corning #354232) laminin on ice.
2. Transfer 10 mL of D-PBS (ATCC 30-2200) to a 15 mL conical tube.
3. Add 100 uL stock laminin to the 15 mL conical tube with D-PBS and mix thoroughly.
4. Added diluted laminin to vessels at a volume of approximately 1 mL per 10 cm2. For example, use 7.5 mL diluted laminin for a 75cm2 flask.
5. Tilt plate to ensure entire surface is coated.
6. Place in a humid cell culture incubator at 37C for 1-24 hours.

Immediately prior to seeding cells, aspirate off the laminin coating solution and discard. Do not allow the plates to dry out.

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). pH (7.0 to 7.6).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Subculturing procedure:

Culture vessels must be pre-coated with laminin prior to seeding with cells.

Coating procedure (perform all steps in a biosafety cabinet)

1. Thaw (Corning cat#354232) laminin on ice.
2. Transfer 10 mL of D-PBS (ATCC 30-2200) to a 15 mL conical tube.
3. Add 100 µL stock laminin to the 15 mL conical tube with D-PBS and mix thoroughly.
4. Added diluted laminin to vessels at a volume of approximately 1 mL per 10 cm^2 . For example, use 7.5 mL diluted laminin for a 75 cm^2 flask.
5. Tilt plate to ensure entire surface is coated.
6. Place in a humid cell culture incubator at 37°C for 1-24 hours.

Immediately prior to seeding cells, aspirate off the laminin coating solution and discard. Do not allow the plates to dry out.

1. Passage cells when the culture has reached approximately 70% to 80% confluence.
2. Pre-coat new culture vessels with laminin, if necessary.
3. Warm Accutase (StemCell Technologies cat# 07920) and complete growth media to room temperature.
4. For each flask, carefully aspirate the spent media without disturbing the

monolayer.

5. Rinse the cell layer one time with 3 to 5 mL D-PBS (ATCC 30-2200) to remove residual medium.
6. Add room temperature Accutase (1 to 2 mL for every 25 cm^2) to each flask.
7. Gently rock each flask to ensure complete coverage of the Accutase solution over the cells, and then aspirate the excess fluid off of the monolayer.
8. Observe the cells under the microscope.
9. 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.
10. Transfer the dissociated cells to a sterile centrifuge tube.
11. Centrifuge the cells at 200 x g for 5 minutes.
12. Aspirate neutralized dissociation solution from the cell pellet and resuspend the cells in 2 to 8 mL fresh, pre-warmed, complete growth medium.
13. Count the cells and seed new culture flasks at a density of 5 x 10^4 viable cells per cm2. Prior to seeding, aspirate the coating and discard the coating laminin solution from the vessel.
14. Place newly seeded flasks in a 37°C, 5% CO_2 incubator for at least 24 to 48 hours before processing the cells further.
15. Perform a complete medium change every 3-4 days.

Reagents for cryopreservation:

Complete growth media containing 10% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: HCM-BROD-0761-C71 (ATCC PDM-499)

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 2025-12-05

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