BT142 mut/-

ACS-1018™

Description

BT142 mut/- is a glioma brain tumor stem cell line derived from the left frontal lobe of a 38-year-old, White male. BT142 mut/- contains a homozygous IDH1 R132H mutation, which originated from a heterozygous IDH1 R132H BT142 cells. The cells grow as phase-bright, smooth spheres. These neural cells can be used for your cancer, neuroscience, stem cell, and toxicology research.

Organism: *Homo sapiens*, human
Cell Type: neural cell
Tissue: Brain
Age: 38 years
Gender: Male
Morphology: Neurosphere
Growth properties: Suspension
Disease: Oligoastrocytoma; Grade III

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.

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: Media: NeuroCult NS-A Proliferation kit (Catalog No. 5751, Stem

Cell Technologies)

Alternate Media: DMEM/F12 (1:1) (Catalog No. 30-2006, ATCC) with an additional 0.9% glucose, 4 mM L-glutamine (Catalog No. 30-2214, ATCC), 25 µg/mL insulin, 100 µg/mL transferrin, 20 nM progesterone, 15 µM putrescine and 30 nM selenite

To make the complete growth medium, add the following supplements to either options of the base medium (see above):

- 20ng/mL recombinant human Epidermal Growth Factor (EGF, Catalog No. 100-15, PeproTech)
- 100 ng/mL recombinant human Platelet-Derived Growth Factor-AA (PDGF-AA, Catalog No. 100-13A, PeproTech)
- 20 ng/mL recombinant human Fibroblast Growth Factor (R&D Systems, Catalog No. 233-FB)
- 2 µg/mL heparan sulfate (Catalog No. H3149, Sigma)

Handling Procedure:

To insure 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 –80°C. Storage at –80°C will result in loss of viability.

1. The cells are cryopreserved as neurospheres and should be thawed as clusters. Do not break apart the neurospheres into a single-cell suspension.
2. Quickly 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.
3. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
4. Transfer the contents of the vial to a centrifuge tube containing 10 mL of complete culture medium.
5. Centrifuge the cells at 200 x g for 10 minutes.
6. Aspirate supernatant and resuspend the cells in 15 mL of complete culture medium and dispense into a 75 cm^2 flask.
7. The cells may take a few weeks to recover from cryopreservation. Viable neurospheres are semi-transparent and phase contrast bright with smooth outer surfaces.

Subculturing procedure:

The cells grow as phase-bright, smooth spheres. The neurospheres should not get too big, ragged or dark as this is a sign of unhealthy, dying cells. The cells should be passaged when the neurospheres are about 200-400 µm in size.

Volumes used in this protocol are for 75 cm^2 flask.

1. Harvest and collect the entire cell suspension from the culture flask into a 15 mL tube.
2. Centrifuge at 200 x *g* for 10 minutes.
3. Aspirate supernatant, leaving approximately 200 µL to cover the pellet.
4. Add 1 mL of complete culture medium.
5. Triturate cells with a P1000 micropipette set to 800 µL by pipetting up and down 40 times or until the cells appear to be in a single cell suspension.
6. Add 8 mL of complete culture medium and centrifuge at 200 x *g* for 10 minutes.
7. Aspirate supernatant and resuspend the cells in 2 mL of complete culture medium.
8. Count viable cells using trypan blue exclusion assay on a hemacytometer.
9. Seed single cells between ranges of 8 x 10^3 to 2 x 10^4 cells/cm^2.

Note: If accurate cell count is necessary, Accumax (Catalog No. AM105, Innovative Cell Technologies) can be used; however, the cells may take some time to recover from an enzymatic dissociation.

Culture maintenance: Replace medium once per week, or as required if media looks depleted, by replacing 5 mL with fresh complete culture medium (volume for 25 cm^2 flask).

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: BT142 mut/- (ATCC ACS-1018)

References

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

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA

US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor