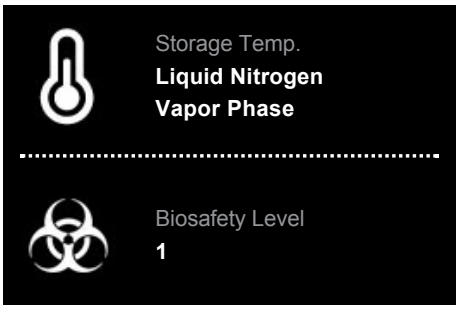
ATCC

Product Sheet

BT142 mut/- (ATCC® ACS-1018™)

Please read this FIRST



Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

There are two options for the base medium for this cell line.
Option 1: NeuroCult NS-A Proliferation kit (Catalog No. 5751, Stem Cell Technologies)
Option 2: 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. H9902, Sigma)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: BT142 mut/- (ATCC® ACS-1018™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

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

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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.

Handling Procedure for Frozen Cells

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.

Handling Procedure for Flask Cultures

Replace medium once per week, or as required if media looks depleted, by replacing 5 mL with fresh complete culture medium (volume for 75 cm^2 flask).

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.

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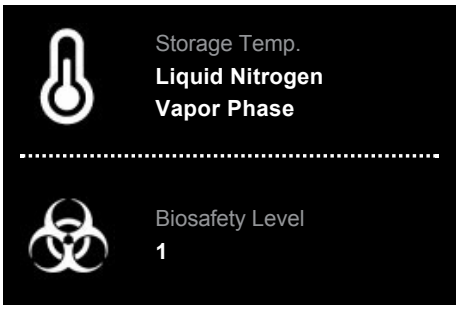

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5. Triturate cells with a P1000 micropipette set to 800 µL by pipetting up and down 40 times or until the cells appear to be in a single cell suspension.
6. Add 8 mL of complete culture medium and centrifuge at 200 x *g* for 10 minutes.
7. Aspirate 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.

Cryopreservation Medium

Complete growth medium described above supplemented with 10% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

Point mutations in isocitrate dehydrogenase I (IDH1) and IDH2 are found in majority of grade II and III gliomas. R132H is the most common IDH1 substitution found in gliomas.
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.
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 200 to 400 µm in size.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.