



BT301

CRL-3415™

Description

Organism: *Homo sapiens*, human

Tissue: Brain

Age: 48 years

Gender: Male

Morphology: bright smooth spheres

Growth properties: Mixed: suspension and aggregate

Disease: Glioblastoma

Cells per vial: Approximately 2.0 to 3.0 x 10⁶

Volume: 1.0 mL

Storage Conditions

Product format: Frozen

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

BT301

CRL-3415

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: NeuroCult Basal Medium w/ proliferation Supplement + 20 ng/mL

BT301

CRL-3415

EGF + 20 ng/mL β -FGF + 2 μ g/mL Heparin sulphate. Protect from light and use complete medium within 10 days.

NeuroCult NS-A Proliferation Kit (Stem Cell Technologies cat# 5751)

- 450 mL NeuroCult Basal Medium (SCT cat# 5750)
- 50 mL NeuroCult Supplement (SCT cat# 5753)

20 μ g/mL EGF: use 1 μ L/1 mL culture medium. To prepare 20 μ g/mL EGF stock solution, aseptically combine:

- 100 μ g EGF (PeproTech cat# 100-15)
- 1 mL PBS (ATCC 30-2200)

Then further dilute by adding 4 mL PBS/0.1% BSA

20 μ g/mL β -FGF: use 1 μ L/1 mL culture medium. To prepare 20 μ g/mL EGF stock solution, aseptically combine:

- β -FGF (PeproTech cat# 100-18B)
- 1 mL 5 mM Tris PH 7.6

Then further dilute by adding 4 mL PBS/0.1% BSA

Store in working aliquots at -20°C . EGF and β -FGF is stable for 1 year when prepared and stored as directed. Do not repeat freeze thaw.

2 mg/mL Heparin Solution (Stem Cell Technologies cat# 07980): use 1 μ L/1 mL culture medium. Store solution at 4°C .

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 -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.

BT301

CRL-3415

3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. 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:

Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 2.0 X 10⁵ cells/mL and maintain between 1.0 X 10⁵ and 3.0 X 10⁶ cells/mL.

Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density).

Reagents for cryopreservation: Complete culture medium (with or without EGF/ β -FGF/Heparin) + 10% DMSO (ATCC 4-X). Store in liquid nitrogen vapor phase.

Notes

Neurospheres must be mechanically broken up when they get too large (they form ragged edges and dark areas within the neurospheres). Do not dispense cells into single cell suspension during this process, but just reduce in size of clusters to maintain a healthy state.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: BT301 (ATCC CRL-3415)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

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 or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

BT301

CRL-3415

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2024-10-25

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

BT301

CRL-3415

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
