BT94

CRL-3412™

Description

Organism: *Homo sapiens*, human
Tissue: Brain
Age: 60 years
Gender: Female
Morphology: bright smooth spheres
Growth properties: Mixed: suspension and aggregate
Disease: Glioblastoma Multiforme
Cells per vial: Approximately 2.0 to 3.0 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

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 EGF + 20 ng/mL β-FGF + 2 µg/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 µg/mL EGF: use 1 µL/1 mL culture medium. To prepare 20 µg/mL EGF stock solution, aseptically combine:

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

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

20 µg/mL β-FGF: use 1 µL/1 mL culture medium. To prepare 20 µg/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 µL/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.

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 cm^2 or a 75 cm^2 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% CO_2 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 10^5 cells/mL and maintain between 1.0 X 10^5 and 3.0 X 10^6 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)

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: BT94 (ATCC CRL-3412)

References

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

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