D556

CRL-3633™

Description

D556 is a pediatric medulloblastoma cell line exhibiting clustered suspension morphology that was isolated from a 7 year old female patient.
Organism: *Homo sapiens*, human
Tissue: Brain
Age: 7 years
Gender: Female
Morphology: Rounded; Aggregated clusters
Growth properties: Adherent
Disease: Medulloblastoma
Cells per vial: Approximately 2.0 to 3.0 x 10^6

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:

The base medium for this cell line is Dulbecco's Modified Eagle's Medium (ATCC 30-2002). To make the complete growth medium, add the following components to the base medium:

- Fetal bovine serum (FBS; ATCC 30-2020) to a final concentration of 10%
- L-Glutamine (ATCC 30-2214) to a final concentration of 2 mM

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 to 400 x g for 8 to 12 minutes.
4. Carefully aspirate the supernatant and discard, leaving the cell pellet.
5. Gently resuspend the cell pellet in 15 mL fresh pre-warmed complete growth medium, and transfer cell suspension into a vented, T75 flask.
6. Place the flask in a 37°C incubator with 5% CO_2 .

Subculturing procedure:

Re-seed cells at log phase every 2-5 days when there are numerous, healthy-appearing clusters present in suspension. Pre-warm fresh growth medium prior to use. Swirl the flask gently to evenly distribute cells in medium.

Cultures can be maintained by addition or replacement of fresh medium. Do not over dilute cell culture, it is recommended to add equal volumes of fresh media and perform full changes via centrifugation if media appears acidic.

1. For acidic cultures, centrifuge cells for 5-12 min at 170 to 400 x g.

2. Discard spent media and re-suspend cell pellet in pre-warmed fresh complete growth media.
3. Pipette cells gently to break aggregates.
4. Add the cell suspension to a vented flask.
5. Place the flask in a 37°C incubator with 5% CO2.

Note: Due to large, tight clusters formed by these cells, it may be difficult to accurately measure cell number and viability. Cultures can be maintained by the addition of fresh medium when there are numerous, healthy-appearing clusters present in suspension and pH of medium has decreased. Alternatively, cultures can be established by centrifugation with subsequent resuspension in fresh medium.

Reagents for cryopreservation: Complete Culture Medium + 5% DMSO (ATCC 4-X)

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: D556 (ATCC CRL-3633)

References

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

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