DG44

CRL-3634™

Description

DG44 is a CHO derivative which is dihydrofolate reductase (DHFR)- deficient: Both alleles of the dhfr gene have been deleted.
Organism: *Cricetulus griseus*, hamster, Chinese
Tissue: Ovary
Gender: Female
Morphology:

Epithelial-like

Growth properties: Adherent
Cells per vial: ≥ 2.0 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:

HyClone™ MEM Alpha Modification with nucleosides and deoxynucleosides (Cytiva catalog # SH30265.01).

- Fetal bovine serum (ATCC 30-2020) to a final concentration of 10%, not heat inactivated

Handling Procedure:

To ensure 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 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 growth medium. and centrifuge at approximately 150-400xg for 8 to 12 minutes (300 x g for 8 minutes).
4. Resuspend cell pellet with the recommended complete medium (see the specific Certificate of Analysis for the culture recommended dilution ratio) and dispense into a 25 cm^2 or a 75 cm^2 culture flask. Cultures can be established between 1.0 x 10^4 and 3.0 x 10^4 viable cells/cm^2. 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.

Note: These cells lack dihydrofolate reductase (DHFR) activity due to deletion of both dhfr gene alleles. To select DHFR+ transformants cells should be grown in Alpha MEM without nucleosides and deoxynucleosides (e.g., from Hyclone or GE

Healthcare) and supplemented with 10% dialyzed fetal bovine serum (e.g.,from Biotechne R&D Systems (formerly Atlanta Biologicals).

Subculturing procedure:

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Corning® T-75 flasks (catalog #430641) are recommended for subculturing this product.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with PBS (ATCC 30-2200) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA for Primary Cells (ATCC PCS-999-003) solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Centrifuge cell suspension at 150 to 200 xg for 8 to 12 minutes to remove dissociation agent.
6. Discard supernatant and resuspend cell pellet in fresh complete culture media.
7. Add appropriate aliquots of the cell suspension to new culture vessels.
8. Incubate cultures at 37°C.

Interval: Subculture at 80-95% confluence. Maintain cultures at a cell concentration between 8.0 X 10^3 and 2.0 X 10^4 cell/cm^2.

Subcultivation Ratio//Subculture seeding density: A subcultivation ratio of 1:4 to 1:8 is recommended/or subculture at a seeding density of 8.0 x 10^3 to 2.0 x 10^4 viable cells/cm^2

Medium Renewal: 2 to 3 times per week

Reagents for cryopreservation:

Complete Culture Medium + 5% DMSO

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: DG44 (ATCC CRL-3634)

References

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

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Revision

This information on this document was last updated on 2025-11-05

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