



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: $\geq 2.0 \times 10^6$

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% CO₂

Handling Procedures

Complete medium:

The base medium for this cell line is HyClone™ MEM Alpha Modification with nucleosides and deoxynucleosides (Cytiva catalog # SH30265.01). 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%

Handling Procedure:

Volumes used in this protocol are for 75 cm² 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 catalog # 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 catalog # 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.
Cultures can be established between 1.0×10^4 and 3.0×10^4 viable cells/cm².
8. Incubate cultures at 37°C.

Interval: Subculture at 80-95% confluence. Maintain cultures at a cell concentration between 8.0×10^3 and 2.0×10^4 cell/cm².

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended

Medium Renewal: 2 to 3 times per week

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)).

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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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