

Data Sheet

PAGE 1/3

BCRJ Code: 0068**Cell Line:** CHO/dhFr-**Species:** Cricetulus griseus**Vulgar Name:** Chinese Hamster**Tissue:** Ovary**Morphology:** Epithelial**Disease:** Normal**Growth Properties:** Adherent**Sex:** Female**Applications:** This cell line is a suitable transfection host.**Biosafety:** 1**Additional Info:**

The CHO/dhFr- cell line lacks the enzyme dihydrofolate reductase (DHFR) which is necessary for purine synthesis. In the absence of exogenous purines, this enzyme is required for growth. Hence the CHO/dhFr- cell line has growth requirements for hypoxanthine (or adenine), glycine and thymidine. However, the cells will continue to grow without the addition of hypoxanthine and thymidine because the cells can obtain sufficient from the medium. Alpha MEM minus does not contain the ribonucleosides or the deoxyribonucleosides, and will not support the growth of CHO/dhFr- or its serum free adaptation as neither contains the gene to produce DHFR.

Culture Medium:

Iscove's modified Dulbecco's medium with 4 mM L-glutamine, 0.1 mM hypoxanthine, 0.016 mM thymidine, 0.002mM Methotrexate (Amethopterin) and 10% of fetal bovine serum.



Subculturing:

Volumes used in this protocol are for 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. T-75 flasks are recommended for subculturing this product. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA 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. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Subculturing Medium Renewal:

Every 2 to 3 days

Subculturing Subcultivation Ratio:

1:4 to 1:8

Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

SAFETY PRECAUTION: It is highly recommended that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submerged 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

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. For cells that are sensitive to DMSO it is recommended that the cryoprotective agent be removed immediately. 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. Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).
5. Incubate the culture in an appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

NOTE: 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 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).

References:

Shulman T, et al. An antibody reactive with domain 4 of the platelet-derived growth factor beta receptor allows BB binding while inhibiting proliferation by impairing receptor dimerization. *J. Biol. Chem.* 272: 17400-17404, 1997. PubMed: 9211881

Morel-Kopp MC, et al. A three amino acid deletion in glycoprotein IIIa is responsible for type I glanzmann's thrombasthenia: importance of residues I1e325Pro326Gly327 for beta 3 integrin subunit association. *Blood* 90: 669-677, 1997. PubMed: 9226167

Libyh MT, et al. A recombinant human scFv anti-Rh(D) antibody with multiple valences using a C-terminal fragment of C4-binding protein. *Blood* 90: 3978-3983, 1997. PubMed: 9354666

Depositors:

Leda dos Reis Castilho, Universidade Federal do Rio de Janeiro

ATCC:

CRL-9096