BCRJ Code:	0067
Cell Line:	CHO 1-15 [subscript 500]
Species:	Cricetulus griseus
Vulgar Name:	Chinese Hamster
Tissue:	Ovary
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Sex:	Female
Age/Ethinicity:	ADULT /
Products:	human tissue plasminogen activator (tPA, t-PA)
Biosafety:	1
Addtional Info:	Expresses human tissue plasminogen activator (t-PA) in eukaryotic DHFR deficient cell lines grown in selective media (glycine, hypoxanthine, thymidine deficient). (Goeddel DV, et al. Human tissue plasminogen activator. US Patent 4,766,075 dated Aug 23 1988) Contains a EcoRI/BamHI fragment of about 2.2 kb containing t-PA and a 1.7 kb SacII fragment containing wild-type dihydrofolate reductase under SV40 control. (Goeddel DV, et al. Human tissue plasminogen activator. US Patent 4,766,075 dated Aug 23 1988)
Culture Medium:	Ham's F12 medium suplemented with 10 % 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:	1:5 to 1:10
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 strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris. 1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize 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 its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions. 3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes. 4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio). 5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).
References:	Goeddel DV, et al. Human tissue plasminogen activator. US Patent 4,766,075 dated Aug 23 1988 Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), ATCC Quality Control Methods for Cell Lines. 2nd edition, Published by ATCC. Caputo, J. L., Biosafety procedures in cell culture. J. Tissue Culture Methods 11:223-227, 1988. Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) Laboratory Safety: Principles and Practice. Second edition, ASM press, Washington, DC. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm
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