

Code:	0050
Cell Line:	BHK-21 [C-13]
Species:	Mesocricetus auratus Vulgar Name: Hamster; Golden Syrian Hamster
Tissue:	Kidney
Morphology:	Fibroblast
Disease:	Normal
Growth Properties:	Adherent
Age Ethnicity:	1 Day old; newborn
Applications:	The World Organization for Animal Health (OIE) uses these cells for routine diagnosis of rabies. Used extensively for virus replication studies i.e. poliovirus, rabies, foot and mouth disease, VSV (Indiana strain), herpes simplex, Ad25 and arboviruses.
Virus Susceptibility:	Human adenovirus 25 Reovirus 3 Vesicular stomatitis virus Human poliovirus 2
Biosafety:	1
Culture Medium:	Dulbecco's modified Eagle's medium with 2mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 1.0 g/L glucose and supplemented with 5 mM HEPES, 90%; fetal bovine serum, 10%.

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. Doubling time: ca. 32-50 hours 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.

Medium Renewal: 1 to 2 times per week

Subcultivation ratio: 1:2 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 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 Oring 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:

Drayna D, et al. Genetic mapping and diagnosis of haemophilia A achieved through a BclI polymorphism in the factor VIII gene. *Nature* 314: 738-740, 1985. PubMed: 2986011 Kazazian HH Jr., et al. Restriction site polymorphism in the phosphoglycerate kinase gene on the X chromosome. *Hum. Genet.* 66: 217-219, 1984. PubMed: 6325324 Macpherson I, Stoker M. Polyoma transformation of hamster cell clones--an investigation of genetic factors affecting cell competence. *Virology* 16: 147-151, 1962. PubMed: 14468055 Macpherson, et al. Polyoma transformation of hamster cell clones - an investigation of genetic factors affecting cell competence. *Virology* 14: 359-370, 1961. Macpherson I. Characteristics of a hamster cell clone transformed by polyoma virus. *J. Natl. Cancer Inst.* 30: 795-815, 1963. Deleersnyder V, et al. Formation of native hepatitis C virus glycoprotein complexes. *J. Virol.* 71: 697-704, 1997. PubMed: 8985401 Yang TT, et al. Quantification of gene expression with a secreted alkaline phosphatase reporter system. *BioTechniques* 23: 1110-1114, 1997. PubMed: 9421645 Hussain MA, et al. POU domain transcription factor brain 4 confers pancreatic alpha-cell-specific expression of the proglucagon gene through interaction with a novel proximal promoter G1 element. *Mol. Cell. Biol.* 17: 7186-7194, 1997. PubMed: 9372951 You M, et al. ch-IAP1, a member of the inhibitor-of-apoptosis protein family, is a mediator of the antiapoptotic activity of the v-Rel oncoprotein. *Mol. Cell. Biol.* 17: 7328-7341, 1997. PubMed: 9372964 Jelachich ML, Lipton HL. Theiler's murine encephalomyelitis virus kills restrictive but not permissive cells by apoptosis. *J. Virol.* 70: 6856-6861, 1996. PubMed: 8794327 Schnell MJ, et al. The minimal conserved transcription stop-start signal promotes stable expression of a foreign gene in vesicular stomatitis virus. *J. Virol.* 70: 2318-2323, 1996. PubMed: 8642658 Chang YE, et al. Properties of the protein encoded by the UL32 open reading frame of herpes simplex virus 1. *J. Virol.* 70: 3938-3946, 1996. PubMed: 8648731 Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. Sydney, NSW, Australia:Standards Australia;Standards Australia AS ISO 10993.5-2002. Biological evaluation of medical devices-- Part 5: Tests for in vitro cytotoxicity. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 10993-5:1999.

Depositors:

Leda dos Reis Castilho, Universidade Federal do Rio de Janeiro

ATCC:

CCL-10