BCRJ Code:	0354
Cell Line:	AML12
Species:	Mus musculus
Vulgar Name:	Mouse, Cd-1
Tissue:	Liver
Cell Type:	Hepatocyte
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	3 Month /
Derivation:	The AML12 (alpha mouse liver 12) cell line was established from hepatocytes from a mouse (CD1 strain, line MT42) transgenic for human TGF alpha.
Applications:	This cell line is a suitable transfection host.
Tumor Formation::	NO
Products:	Albumin; human transforming growth factor alpha (TGF alpha); mouse TGF alpha
Biosafety:	1

Addtional Info:	By electron microscopy, these cells exhibit typical hepatocyte features such as peroxisomes and bile canalicular like structure. AML12 cells retain the capacity to express high levels of mRNA for serum (albumin, alpha 1 antitrypsin and transferrin) and gap junction (connexins 26 and 32) proteins, and contain solely isoenzyme 5 of lactate dehydrogenase. The cells express high levels of human TGF alpha and lower levels of mouse TGF alpha. Expression of liver specific proteins decreases with time in culture, but is reactivated by growing the cells in serum free medium.
Culture Medium:	1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 0.005 mg/mL insulin, 0.005 mg/mL transferrin, 5 ng/mL selenium, and 40 ng/mL dexamethasone 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. 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:	2 to 3 times a week
Subculturing Subcultivation Ratio:	1:4 to 1:6
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: Is highly recommend 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 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 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 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 a 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:	Wu JC, et al. Establishment and characterization of differentiated, nontransformed hepatocyte cell lines derived from mice transgenic for transforming growth factor alpha. Proc. Natl. Acad. Sci. USA 91: 674-678, 1994. PubMed: 7904757 Dumenco L, et al. Introduction of a murine p53 mutation corresponding to human codon 249 into a murine hepatocyte cell line results in growth advantage, but not in transformation. Hepatology 22: 1279-1288, 1995. PubMed: 7557882
Depositors:	RICARDO BENTES DE AZEVEDO - UNIVERSIDADE DE BRASILIA
ATCC:	CRL-2254
Cellosaurus:	CVCL_0140