Data Sheet

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: 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:	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
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