

Code:	0291
Cell Line:	C3A [HepG2/C3A, derivative of Hep G2]
Species:	Homo sapiens Vulgar Name: Human
Tissue:	Liver
Morphology:	Epithelial
Disease:	Hepatocellular Carcinoma
Growth Properties:	Adherent
Sex:	Male
Age Ethnicity:	15 YEARS ADOLESCENT, CAUCASIAN
Derivation:	C3A is clonal derivative of Hep G2 that was selected for strong contact inhibition of growth, high albumin production, high production of alpha fetoprotein (AFP) and ability to grow in glucose deficient medium.
Applications:	This cell line is a suitable transfection host.
DNA Profile:	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,13 D7S820: 10 THO1: 9 TPOX: 8,9 vWA: 17
Tumor Formation:	No, in immunosuppressed mice Yes, in semisolid medium
Products:	alpha-fetoprotein (alpha fetoprotein); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin; (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; complement (C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein)
Biosafety:	1

Additional info: As the cells become confluent, there is a marked reduction in AFP secretion and an increase in albumin secretion. Gluconeogenesis activity is strongly oxygen dependent. The cells have nitrogen metabolizing activity comparable to perfused rat livers. There is no evidence of a Hepatitis B virus genome in this cell line.

Culture Medium: Dulbecco's Modified Eagle's Medium (DMEM) containing 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and fetal bovine serum to a final concentration of 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. 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.

Medium Renewal: Twice per week

Subcultivation ratio: 1:3 to 1:6 is recommended

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:

Kelly JH. Permanent human hepatocyte cell line and its use in a liver assist device (LAD). US Patent 5,290,684 dated Mar 1 1994

Depositors:

Ana Cecília Oliveira - Fundação Oswaldo Cruz

ATCC:

CRL-10741