Hep-66.3A

400206

GENERAL INFORMATION

Description

The Hep-66.4A hepatoma cell line is derived from a mouse liver tumor, specifically from the C57BL/6J mouse strain. This cell line is characterized by its hepatocytic origin, confirmed through intermediate filament protein analysis. Hep-66.4A expresses simple keratins K8 and K18, which are typical of normal liver cells, as well as vimentin and keratin K19 to varying degrees. These protein patterns confirm the hepatocytic nature of the cell line and its classification as a hepatoma line.
The Hep-66.4A cell line displays a predominantly epithelial morphology, reflecting its origin from hepatocytes. This morphological phenotype is consistent with its protein expression profile. DNA fingerprint analysis of Hep-66.4A did not reveal any major structural abnormalities, indicating a degree of genomic stability. However, some changes in the relative intensities of specific bands were observed with increasing passage numbers, suggesting minor genomic variability over extended culture periods.
Despite the absence of detectable p53 mutations in the primary mouse liver tumors, aberrations were found in some hepatoma lines during in vitro propagation. The Hep-66.4A cell line was analyzed for mutations in the p53 and c-Ha-ras genes. The absence of detectable mutations in the p53 gene in this line during early passages suggests a stable genetic background. This cell line serves as a valuable model for studying hepatocellular carcinoma, providing insights into the cellular and molecular mechanisms underlying liver tumorigenesis.

Tissue	Liver
Disease	Hepatocellular carcinoma

Synonyms

- HEP-66.3A
- 66.3A

CLASSIFICATION

Organism	Mouse
Sex	Female
Growth properties	Adherent
Biosafety level	1

CHARACTERISTICS

Morphology	Epithelial-like
Tumourigenic	Yes, in B6C3F1 mice

IDENTIFIERS

Citation

Hep-66.3A (Cytion catalog number 400206)

NCBI TaxID	10090
Cellosaurus	CVCL_5771

Cytion GmbH
Nikola-Tesla-Str. 3
69124 Heidelberg
Tel: +49 (0)6221 405780
info@cytion.com
cytion.com

Registered office Heidelberg
VAT ID: DE283292917
Local court Mannheim - HRB 714394
CEO / Geschäftsführer: Jonathan Steubing

Banking information
Sparkasse Heidelberg
IBAN: DE35 6725 0020 0009 2076 86
BIC: SOLADES1HDB

EXPRESSION AND SUSCEPTIBILITY

Protein expression

Keratin 8, Keratin 18, Vimentin

Mutational profile

P53 wt

HANDLING AND CULTURE

Culture medium

DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 10% FBS

Dissociation reagent	Accutase

Subculturing

Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Fluid renewal	Every 3 to 5 days

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.
To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

Freeze medium	As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

DONOR

Breed / subspecies	C57BL/6J
Donor age	Adult

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Disclaimer

Our cells are provided for in vitro laboratory research purposes exclusively and are not intended for clinical or diagnostic use, nor are they to be administered to humans or used for veterinary purposes. Users must adhere to all applicable guidelines and regulations for the handling and use of these cells in a research setting.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Material Transfer Agreement required	Yes