**Product sheet**

# Hep-56.1B Cells | 400102

## General information

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| **Description** | The Hep-70.4 hepatoma cell line is derived from a mouse liver tumor, specifically from the C57BL/6J mouse strain. This cell line is notable for its mutations in the p53 gene, which were identified at different passages during in vitro propagation. At passage number 8, a weak additional signal was detected in the single-strand conformation polymorphism (SSCP) analysis, indicating the presence of a p53 mutation. By passage number 38, two distinct p53 point mutations were identified: a G:C to C:G transversion at codon 135 and a C:G to G:C transversion at codon 138 of exon 5. These mutations led to amino acid changes from alanine to proline and cysteine to tryptophan, respectively.<br><br>The Hep-70.4 cell line displays a morphological phenotype that varies significantly during its propagation. Some sublines exhibit an epithelial morphology, while others show a fibroblast-like appearance. This heterogeneity reflects the complex nature of the cell line and its adaptability under different culture conditions. The presence of both normal and mutated p53 alleles in the early passages suggests that the mutations confer a selective growth advantage, leading to the predominance of mutated clones over time.<br><br>Intermediate filament protein analysis of the Hep-70.4 cell line revealed the expression of 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 origin of the cell line and its classification as a hepatoma line. The genomic stability of Hep-70.4 was further assessed through DNA fingerprint analysis, which did not reveal any major structural abnormalities, although changes in the relative intensities of certain bands were observed with increasing passage numbers. |
| **Organism** | Mouse |
| **Tissue** | Liver |
| **Disease** | Hepatocellular carcinoma |
| **Synonyms** | HEP-56.1B, 56.1B, 56.1b |

## Characteristics

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| **Breed/Subspecies** | C57BL/6J |
| **Age** | Adult |
| **Gender** | Female |
| **Morphology** | Epithelial-like |
| **Growth properties** | Adherent |

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## Regulatory Data

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| **Citation** | Hep-56.1B (Cytion catalog number 400202) |
| **Biosafety level** | 1 |
| **NCBI_TaxID** | 10090 |
| **CellosaurusAccession** | CVCL_5767 |

## Biomolecular Data

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| **Protein expression** | Keratin 8, Keratin 18, Vimentin. |
| **Tumorigenic** | Yes, in C57BL/6J mice |
| **Mutational profile** | P53mut (codon 277 in exon 8 => Arginin -- Threonin). |

## Handling

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| **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) |
| **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. |
| **Seeding density** | $1 \times 10^4$ cells/$cm^2$ |
| **Fluid renewal** | Every 3 to 5 days |

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| **Post-Thaw Recovery** | After thawing, plate the cells at 5 x $10^4$ cells/$cm^2$ and allow the cells to recover from the freezing process and to adhere for at least 24 hours. |
| **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. |
| **Thawing and Culturing Cells** | 1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.<br>2. 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.<br>3. 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.<br>4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.<br>5. 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.<br>6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.<br>7. 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.<br>8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes. |
| **Incubation Atmosphere** | 37°C, 5% $CO_2$, humidified atmosphere. |
| **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. |

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| **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. |

## Quality Control & Molecular Analysis

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