



General information

| Description | Established from the primary hepatocellular carcinoma of C57BL/6J mice. |
|-------------|---|
| Organism | Mouse |
| Tissue | Liver |
| Disease | Hepatocellular carcinoma |
| Synonyms | HEP-56.1C, 56.1C, 56.1c |

Characteristics

| Age | Adult |
|-------------------|-----------------|
| Gender | Female |
| Morphology | Epithelial-like |
| Growth properties | Adherent |

Identifiers / Biosafety / Citation

| Citation | Hep-56.1C (Cytion catalog number 400203) |
|-----------------|--|
| Biosafety level | 1 |

Expression / Mutation

Handling

| Culture Medium | DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 1.5 g/L NaHCO3, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a) |
|-----------------------|---|
| Medium supplements | Supplement the medium with 10% FBS |



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| Passaging solution | 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. |
| Split ratio | A ratio of 1:4 to 1:8 is recommended |
| Seeding density | 1 x 10^4 cells/cm^2 |
| Fluid renewal | Every 3 to 5 days |
| Freezing recovery | After thawing, plate the cells at 5×10^4 cells/cm ² and allow the cells to recover from the freezing process and to adhere for at least 24 hours. |
| Freeze medium | CM-1 (Cytion catalog number 800100) or CM-ACF (Cytion catalog number 806100) |



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Handling of cryopreserved cultures

- 1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
- 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.
- 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.
- 4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
- 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.
- 6. Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium. Optionally, skip centrifugation but remove any remaining freezing medium after 24 hours.
- 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.
- 8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Quality control / Genetic profile / HLA

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.



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STR profile M_18-3: 16

M_4-2: 20.3 **M_6-7**: 17 **M_3-2**: 14 **M_19-2**: 13 **M_7-1**: 26.2 **M_1-1**: 16 **M_8-1**: 16 **M_2-1**: 15 **M_15-3**: 22.3 **M_6-4**: 18 **M_11-2**: 16 **M_1-2**: 19 **M_17-2**: 15 **M_12-1**: 17 **M_5-5**: 17 **M_X-1**: 28 **M_13-1**: 17

Human D4/D8: -