

HT22 Cells | 305158

General information

**Description**

The HT22 cell line, an immortalized subclone derived from HT4 cells of the mouse hippocampus, is pivotal in neuropharmacological research. Originating through the immortalization of mouse neuronal tissues with a temperature-sensitive SV40 T-antigen, HT22 cells offer a unique in vitro model to investigate the mechanisms underlying glutamate-induced cytotoxicity, which plays a significant role in neurodegenerative disorders such as Alzheimer's, Huntington's, and Parkinson's diseases.

HT22 cells exhibit a neuronal phenotype and are highly sensitive to glutamate, an essential excitatory neurotransmitter involved in critical brain functions like cognition, learning, and memory. However, excessive glutamate intake can lead to glutamate toxicity and overexcitation of nerve cells, causing cell damage or death through mechanisms that involve oxidative stress and apoptosis.

HT22 mouse hippocampal cells are employed in neurotoxicity studies, such as those examining the effects of isoflurane exposure, for exploring the chromatin landscape and epigenetic signatures, and to examine the effects of serotonergic input on hippocampal neurogenesis. The latter includes the study of serotonin reuptake inhibitors and their role in antidepressant screening, as well as the impact of serotonin transporter (SERT) glycosylation on neuronal function.

The HT22 cell line, with its well-characterized response to glutamate and its utility in studying the serotonergic system, continues to be a valuable tool in the advancement of neuropharmacology and the development of treatments for a range of neurological disorders.

**Organism** Mouse

**Tissue** Brain, hippocampus

**Synonyms** HT-22

Characteristics

**Morphology** Epithelial

**Growth properties** Adherent

Regulatory Data

**Citation** HT22 (Cytion catalog number 305158)

**Biosafety level** 1

**NCBI\_TaxID** 10090

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**CellosaurusAccession** CVCL\_0321

**GMO Status**

GMO-S1: This murine hippocampal neuronal cell line (HT22) contains a retroviral construct encoding temperature-sensitive SV40 T-Antigen, supporting conditional immortalization. The insert is stably present in neuronal precursor cells. This classification applies only within Germany and may differ elsewhere.

### Biomolecular Data

### Handling

**Culture Medium**

DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO<sub>3</sub>, 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.

**Freeze medium**

As a cryopreservation medium, we use 50% basal medium + 40% FBS + 10% DMSO, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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### 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.
2. Upon receipt, either store the cryovial immediately at temperatures below  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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  $300 \times g$  for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
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.

### Incubation Atmosphere

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Flask Coating

None

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

### Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about  $-150$  to  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  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.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.