Data Sheet

HT-22 Mouse Hippocampal Neuronal Cell Line

Immortalized Cell Line

SCC129

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Product Overview

Glutamate is a major excitatory neurotransmitter used as a signaling molecule between nerve cells and is involved in most aspects of normal brain function including cognition, learning and memory. Various glutamate receptors are found throughout the brain and include NMDA, AMPA/kainate and metabotropic receptors (mGluR).
Normal glutamate levels are essential for normal brain function, however high levels may result in overexcitation of nerve cells, leading to eventual cell damage and/or cell death. Glutamate-induced cytotoxicity has been implicated in neurodegenerative disorders such as Alzheimer's disease, Huntington's disease and Parkinson's disease along with other conditions including spinal cord injury and multiple sclerosis. Therapies that may inhibit or reduce the effects of glutamate activity are thus of great therapeutic interest.

HT-22 is an immortalized mouse hippocampal cell line subcloned from the HT-4 cell line.[1] The parental HT-4 cell line was derived from the immortalization of mouse neuronal tissues with a temperature sensitive SV40 T-antigen.[2] HT-22 is highly sensitive to glutamate and is thus frequently used as a model system to study glutamate-induced toxicity in neuronal cells.

Storage and Handling

HT-22 Mouse Hippocampal Neuronal Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥1 × 10^6 viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for interspecies contamination from rat, Chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for *Mycoplasma* contamination.

Protocol

Thawing Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on normal tissue cultureware surfaces without any additional coating.

   HT-22 Expansion Medium: Cells are thawed and expanded in High Glucose DMEM (D6546), 10% FBS (ES-009-B), 1X L-Glutamine (TMS-002-C) and 1X Penicillin-Streptomycin Solution (TMS-AB2-C).
2. Remove the vial of frozen HT-22 cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor the cells until they are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   Important: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1- or 2-mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of HT-22 Expansion Medium (Step 1 above) to the 15 mL conical tube.

   Important: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   Important: Do not vortex the cells.
7. Centrifuge the tube at 300 *x g* for 2–3 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5–8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 10-15 mL of HT-22 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.
12. The next day, exchange the medium with 10-15 mL of fresh HT-22 Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 70% confluent, they can be dissociated with Accutase® (SCR005) or Trypsin-EDTA (SM-2003-C) and further passaged or, alternatively, frozen for later use.

    Note: Do not let cells grow past 70% confluence as their properties change if they are repeatedly allowed to become confluent. Cells will not die properly if they are repeatedly allowed to grow to confluence.

HT-22 Oxidative Glutamate Toxicity Assay

14. Plate HT-22 cells at 5×10^3 cells/well in a 96-well plate or equivalent number of cells in larger dishes (e.g., 1.5×10^5 cells/35 mm dish; 3×10^5 cells/60 mm dish, etc).
1. Next day, change media to DMEM with 7.5% fetal calf serum and penicillin/streptomycin solution.
2. Treat cells with desired agent at desired timepoints (e.g., 8, 16, 24 hours).
3. Measure cell viability with MTT Cell Growth Assay Kit (CT02) according to manufacturer's kit instructions.

Cryopreservation of Cells

HT-22 Mouse Hippocampal Neuronal Cell Line may be frozen in the expansion medium plus 8-10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

Data Analysis

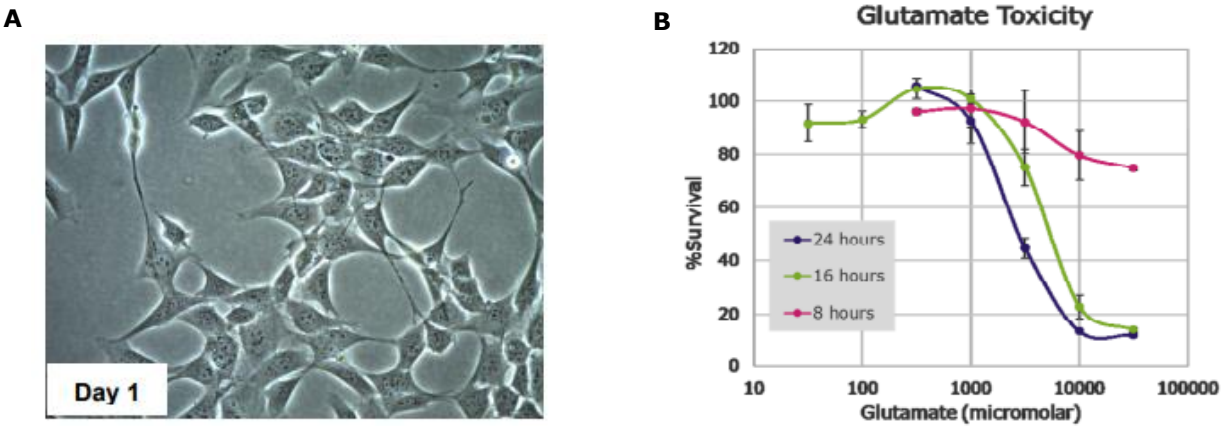

Figure 1. A. Day 1 image after thaw. **B.** Glutamate causes a dose-dependent killing of HT-22 over prolonged exposure. HT22 cell survival upon exposure of various concentrations of glutamate (e.g., 100 µM to 10 mM) at specified timepoints (8, 16, 24 hours). Cell viability was measured via the MTT Cell Growth Assay Kit.

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