GT1-7 Mouse Hypothalamic GnRH Neuronal Cell Line

Immortalized Cell Line

Cat. # SCC116

Pack size: ≥1X10^6 viable cells/vial

Store in liquid nitrogen

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
NOT FOR HUMAN OR ANIMAL CONSUMPTION.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Certificate of Analysis

Background

Gonadotrophin-releasing hormone (GnRH) is a neuropeptide that is synthesized and released from GnRH neurons within the hypothalamus and is required for normal reproductive development and function. The scarcity and scattered distribution of GnRH neurons in the rostral hypothalamus make the biological study of the cells difficult.

GT1-7 is an immortalized mature mouse hypothalamic GnRH neuronal cell line. Immortalized GnRH neurons were generated by introducing a transgene containing the promoter region of the GnRH gene coupled to the coding region of the SV40 T-antigen oncogene into transgenic mice[1]. The resulting anterior hypothalamic tumors were removed from one of the mice and the cells were dissociated and cloned. GT1-7 is a clonal line of mature differentiated GnRH neurons that exhibit high levels of *Gnrh1* mRNA and secrete GnRH in response to depolarization.

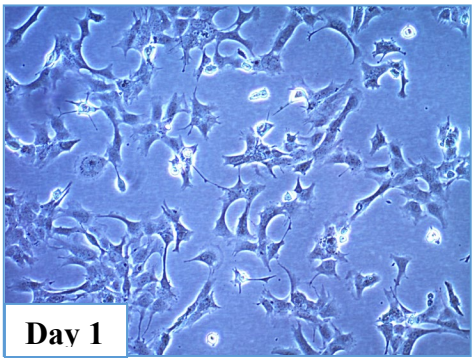
Storage and Handling

GT1-7 Mouse Hypothalamic GnRH 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 ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Mouse/Rat Comprehensive CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Data



References

1. Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL and Weiner RI. (1990) Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. *Neuron* 5(1): 1-10.
2. Liposits Z, Merchenthaler I, Wetsel WC, Reid JJ, Mellon PL, Weiner RI, and Negro-Vilar A. (1991) Morphological characterization of immortalized hypothalamic neurons synthesizing luteinizing hormone-releasing hormone. *Endocrinology* 129(3): 1575-1583.
3. Wetsel WC, Mellon PL, Weiner RI, and Negro-Vilar A. (1991) Metabolism of pro-luteinizing hormone-releasing hormone in immortalized hypothalamic neurons. *Endocrinology* 129(3): 1584-1595.
4. Wetsel WC, Valença MM, Merchenthaler I., Liposits Z., López FJ, Weiner RI, Mellon PL, and Negro-Vilar A. (1992) Intrinsic pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons *Proc. Natl. Acad. Sci. USA* 89(9): 4149-4153.
5. Whyte DB, Lawson MA, Belsham DD, Eraly SA, Bond CT, Adelman JP, and Mellon PL. (1995) A neuron-specific enhancer targets expression of the gonadotropin-releasing hormone gene to hypothalamic neurosecretory neurons. *Mol. Endocrinol* 9(4): 467-477.

SPECIES LEGEND: **H** Human **Ca** Canine **M** Mouse **R** Rat **Rb** Rabbit **B** Bovine **P** Porcine **WR** Most Common Vertebrates

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Protocols

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.

   Cells are thawed and expanded in High Glucose DMEM (Sigma Cat. No. D6546), 10% FBS (EMD Millipore Cat. No. ES-009-B), 2 mM L-Glutamine (EMD Millipore Cat. No. TMS-002-C) and 1X Penicillin-Streptomycin Solution (EMD Millipore Cat. No. TMS-AB2-C).
2. Remove the vial of frozen GT1-7 cells from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells 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 GT1-7 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 GT1-7 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 GT1-7 Expansion Medium. Exchange with fresh medium every two to three days thereafter.
13. When the cells are approximately 90-95% confluent, they can be dissociated with Accutase (EMD Millipore Cat. No. SCR005) or trypsin-EDTA (EMD Millipore Cat. No. SM-2003-C) and further passaged or, alternatively, frozen for later use.

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Subculturing Cells

Note: GT1-7 cells are sensitive to over-trypsinization and to excessively sparse plating. They are also sensitive to overgrowth and to depleted media.

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of GT1-7 cells.
2. Rinse the T75 flask twice with 10 mL 1X PBS. Aspirate after each rinse.
3. Apply 3-5 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 8 mL of GT1-7 Expansion Medium to the plate.
6. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
7. Centrifuge the tube at 300 x g for 3-5 minutes to pellet the cells.
8. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
9. Apply 2 mL of GT1-7 Expansion Medium to the conical tube and resuspend the cells thoroughly.

   IMPORTANT: Do not vortex the cells.
10. Count the number of cells using a hemocytometer.
11. Plate the cells to the desired density (typical split ratio is 1:5 – 1:7).

Cryopreservation of Cells

GT1-7 Mouse Hypothalamic GnRH Neuronal Cell Line may be frozen in the expansion medium plus 8-10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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