GN11 Mouse GnRH-Secreting Neuronal Cell Line

Immortalized Cell Line

Cat. # SCC188

Pack size: ≥1x10^6 viable cells/vial

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

Store in liquid nitrogen

Data Sheet

Background

Gonadotropin-releasing hormone (GnRH) is important for control of reproduction in all mammals, acting to stimulate the synthesis and secretion of follicle-stimulating hormone and luteinizing hormone from the pituitary gland.[1] GnRH is secreted by specialized neurons that migrate from the olfactory bulb to the brain during embryonic development. GnRH neurons are highly sensitive to glucose and insulin, suggestive of their physiological role in sensing the metabolic status in preparation for reproduction.[2] Defects in GnRH neuronal function result in subfertility and developmental abnormalities. The scattered distribution and scarcity of GnRH neurons in the brain complicate the *in vivo* study of these cells and necessitate the utilization of robust *in vitro* cell models.

The GN11 mouse neuronal cell line is an immortalized cell line established as a model of GnRH-secreting neurons.[3] In addition to GnRH expression, GN11 cells display neuron-specific markers including microtubule-associated protein 2 (MAP2) and Tau[4] GN11 cells express insulin receptor[5] and are responsive to steroid hormones.[6] The GN11 GnRH-secreting neuronal cell line has proven a valuable system for exploring the cellular mechanisms of neuroendocrine reproductive physiology.

Source

GMO. The GN11 cell line was derived from a transgenic mouse expressing Simian virus-40 (SV40) large T antigen driven by the human GnRH promoter.[3] An olfactory bulb tumor from one male GnRH promoter-SV40T fusion-expressing transgenic mouse were dispersed and the GN11 cell line was established from serial dilution and cloning from the primary cells.

Quality Control Testing

- Each vial contains ≥ $1X10^6$ 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 inter-species 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.

Storage and Handling

Cells 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.

Representative Data

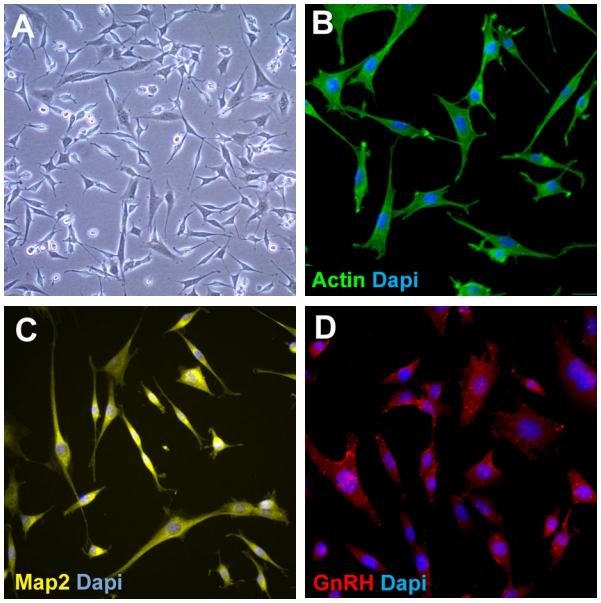

Figure 1. GN11 cells one day after thawing in a T75 flask (**A**). Cells express actin (**B,** P5282), Map2 (**C, A**B5622) and GnRH (**D,** MAB5456). **GnRH** staining appears as intracellular punctae

References

1. Forni PE, Wray S (2012) *Mol Neurobiol* 46(2): 349-360.
2. Roland AV, Moenter SM (2011) *Trends Endocrinol Metab* 22(11): 443-449.
3. Radovick S et al. (1991) *Proc Natl Acad Sci USA* 88(8): 3402-3406.
4. Zhen S et al. (1997) *J Biol Chem* 272 (19): 12620-12625.
5. DiVall SA, et al. (2007) Mol Cell Endocrinol 270(1–2):64–72.
6. Novaira HJ, et al. (1994) Horm Behav 28(4):520-529.

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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.

   GN11 Expansion Medium: Cells are thawed and expanded in DMEM medium (Cat. No. SLM-120-B) supplemented with 7% FBS (Cat. No. ES-009-B) and 3% Newborn Calf Serum (Sigma N4637).
2. Remove the vial of frozen GN11 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 GN11 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 15 mL of GN11 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.

Subculturing Cells

Note: Passage when cells are at 80% confluency. Do not allow cells to grow to 100% confluency.

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of GN11 cells.
2. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 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 5-7 mL of GN11 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 2-5 mL of GN11 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 1:6.

Cryopreservation of Cells

GN11 Mouse GnRH-Secreting Neuronal Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

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