

## Data Sheet

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<b>BCRJ Code:</b>	0095
<b>Cell Line:</b>	GT1-7
<b>Species:</b>	Mus musculus
<b>Vulgar Name:</b>	Mouse
<b>Tissue:</b>	Brain
<b>Cell Type:</b>	Fibroblast
<b>Morphology:</b>	Fibroblast
<b>Growth Properties:</b>	Adherent
<b>Derivation:</b>	The GT1-7 line was immortalized by expression of SV40 T antigen oncogene in hypothalamic GnRH neurons.
<b>Products:</b>	GnRH - Gonadotropin-Releasing Hormone.
<b>Biosafety:</b>	2
<b>Additional Info:</b>	GT1-7 cell line is sensitive to trypsin and low confluence.
<b>Culture Medium:</b>	Dulbecco's modified Eagle's medium with 4.5 g/L glucose and 10% of fetal bovine serum.

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### Subculturing:

Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. Doubling time: 36 hours NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

### Subculturing Medium Renewal:

Every 2 to 3 days

### Subculturing Subcultivation Ratio:

1:5 to 1:7

### Culture Conditions:

Atmosphere: air, 95%; carbon dioxide (CO<sub>2</sub>), 5% Temperature: 37°C

### Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

## Thawing Frozen Cells:

**SAFETY PRECAUTION:** It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately  $125 \times g$  for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

**NOTE:** It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

## References:

Nelson SB, Lawson MA, Kelley CG, Mellon PL (2000) "neuron-specific expression of the rat gonadotropin-releasing hormone gene is conferred by interactions of a defined promoter with the enhancer in GT1-7 cells". *Mol Endocrinol* 14 (9): 1509-22. Mellon, P.L., Windle, J.J., Goldsmith, P., Pedula, C., Roberts, J. and Weiner, R.I. 1990. immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. *Neuron* 5: 1-10. Liposits, Z., Merchenthaler, I., Westel, W.C., Reid, J. J., Mellon, P. L., Weiner, R.I. and Negro-Villar, A. 1991. Morphological characterization of immortalized hypothalamic neurons synthesizing luteinizing hormone-releasing hormone. *Endocrinol.* 129: 1575-1583. Wetsel, W.C., Valença, M.M., Merchenthaler, I. Liposits, Z., López, F.J., Weiner, R.I., Mellon, P.L. and Negro-Villar, A. 1992. Intrinsic pulsatile secretory activity of immortalized luteinizing hormone-releasing hormone-secreting neurons. *Proc. Natl. Acad. Sci. USA* 89:4149-4153. Whyte, D. B., Lawson, M.A., Belsham, D. D., Eraly, S.A. Bond, C.T., Adelman, J. P., and Mellon, P.L. 1995. A Neuron-Specific Enhancer Targets Expression of the Gonadotropin-Releasing Hormone Gene to Hypothalamic Neurosecretory Neurons. *Molecular Endocrinology* 9, 467-477.

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