Data Sheet

# LbetaT2 Mouse Pituitary Gonadotrope Cell Line

Immortalized Cell Line

**SCC412**

**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.

## Background

Gonadotropin-releasing hormone (GnRH) plays a key role in the control of reproduction in mammals. GnRH acts via its receptor, GnRH-R, to trigger the synthesis and release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by the pituitary gonadotropes. In turn, the gonadotropins regulate gametogenesis and steroidogenesis in the gonads. LH and FSH comprise a common glycoprotein hormone α subunit, CGA, and the respective specific β subunits LHβ and FSHβ. The individual β subunits bind noncovalently to the common a subunit to form the mature glycoprotein hormones. *Lhb* gene expression is preferentially induced by high-frequency GnRH pulses, whereas low-frequency pulses favor *Fshb* expression.

## Source

The immortalized LβT2 mouse pituitary gonadotrope cell line was generated by targeted tumorigenesis in transgenic mice carrying the rat LHb regulatory region linked to the SV40 T-antigen oncogene.[1] LβT2 cells express CGA, GnRH-R, and LHb. The cell line responds to pulsatile GnRH stimulation by upregulating *Lhb* and *Gnrhr* and secreting LH. The LβT2 cell line has been widely used as an in vitro model for the study of gonadotropin gene regulation and GnRH signaling.[2,3,4,5] The global mRNA expression profile and a genome-wide atlas of accessible chromatin in GnRH-stimulated LβT2 cells has recently been described[6] and the STR profile has also been reported.[7]

## Short Tandem Repeat (STR) Profile

| | | | |
|---|---|---|---|
| M1-1: | 16, 17 | M8-1: | 16 |
| M1-2: | 19 | M11-2: | 16, 18 |
| M2-1: | 16 | M12-1: | 16, 17 |
| M3-2: | 13 | M13-1: | 16.2 |
| M4-2: | 20.3, 21.3 | M15-3: | 22.3 |
| M5-5: | 13, 17 | M17-2: | 15 |
| M6-4: | 19, 20 | M18-3: | 16, 17 |
| M6-7: | 12 | M19-2: | 12 |
| M7-1: | 26.2 | MX-1: | 25 |

## Quality Control Testing

- LβT2 mouse pituitary gonadotrope cells are verified to be of mouse origin and negative for rat, Chinese hamster, Golden Syrian hamster, human, and non-human primate interspecies contamination as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

## Storage and Handling

LβT2 mouse pituitary gonadotrope cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

## Representative Data

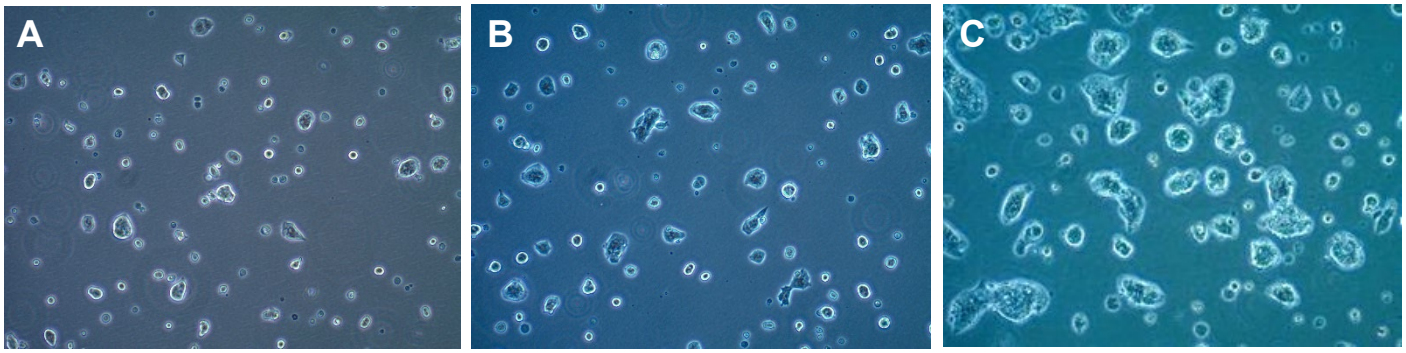


**Figure 1**. Bright-field images of LβT2 cells one (**A**) and two days (**B**) after thaw. LβT2 cells adhere to culture plates and grow as small clumps of cells (**C**).

## Protocols

### Thawing the 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 LβT2 Expansion Medium comprising High Glucose DMEM containing L-glutamine and Sodium Pyruvate (Cat. No. D6429), and 10% FBS (Cat. No. ES-009-B).

2. Remove the vial of frozen LβT2 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 LβT2 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 LβT2 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 the Cells

LβT2 cells adhere to the treated plastic surface of tissue culture plates as small groups of cells. They do not disperse as a single monolayer and will not grow to "confluence". LβT2 cells are sensitive to over-trypsinization and to excessively sparse plating. They are also sensitive to overgrowth and to depleted media.

1. LβT2 cells should be passaged at ~ 70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of LβT2 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase® reagent and incubate in a 37 °C incubator for 3-5 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 5-7 mL of LβT2 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of LβT2 Expansion Medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    **IMPORTANT**: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

### Cryopreservation of the Cells

LβT2 Mouse Pituitary Gonadotrope Cell Line may be frozen in LβT2 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

## References

Please include the appropriate references from 1-5 in your publications that utilize them.


1. Alarid ET, Windle JJ, Whyte DB, Mellon PL. *Development* 1996; 122(10):3319-3329.
2. Turgeon JL, Kimura Y, Waring DW, Mellon PL. *Molecular Endocrinology* 1996; 10(4):439-450.
3. Thomas P, Mellon PL, Turgeon J, Waring DW. *Endocrinology* 1996; 137(7):2929-2989.
4. Alarid ET, Holley S, Hayakawa M, Mellon PL. *Mol Cell Endocrinol* 1998; 140(1-2):25-30.
5. Pernasetti F, Vasilyev VV, Rosenberg SB, Bailey JS, Huang HJ, Miller WL, Mellon PL. *Endocrinology* 2001; 142(6):2284-2295.
6. Ruf-Zamojski F et al. *Front Endocrinol (Lausanne)* 2018; 9:34.
7. Ruf-Zamojski F et al. *J Endocr Soc* 2019; 3(5):902–920.

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