Data Sheet

GLUTag Mouse Colonic Endocrine Cell Line

Cancer Cell Line

SCC652

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

Glucagon-like peptide-1 (GLP-1), an important regulator of glucose dependent insulin secretion, is a peptide hormone produced by enteroendocrine cells.[2] It also plays a role in food intake and gut motility. GLP-1 activity is preserved in individuals with type 2 diabetes, allowing it to be an effective target for drugs to treat diabetes and obesity.

The GLUTag cell line is an effective intestinal endocrine L-cell model derived from colonic tumors of transgenic mice. This cell line secretes a variety of pro-glucagon derived peptides (PGDPs) such as GLI, IRG, and GLP-1.[1] GLUTag cells are among one of the most widely used cell models to understand GLP-1 secretion. GLUTag cells can activate GLP-1 secretion in vivo and have a phenotype that closely mirrors non-immortalized intestinal L-cells with some slight genetic differences. The immunoreactive profile of GLUTag cells also suggests L-cell lineage by being positively immunoreactive to GLP-1 and cholecystokinin, but negative for a large variety of other hormones such as calcitonin, insulin, and gastrin.

Although the GLUTag cell line has murine origins, they have been shown to have a relevant response mechanism that essentially mirrors the GLP-1 secretion response in humans. This means that testing potential therapeutic agents using GLUTag cells has been shown to still have a strong relevance to a human model. The ease-of-use of GLUTag cells becomes quite invaluable when compared to the difficulties of using primary human L-cells.

Source

Derived from an endocrine carcinoma in a mouse expressing SV40 Large T antigen. Fragments of the tumors were subcutaneously propagated in nude mice and individual cells from one of the tumors was isolated and single cell cloned.

Short Tandem Repeat

M18-3: 22	M4-2: 19.3	M6-7: 12, 14	M1-2: 13	M7-1: 29	M1-1: 10,11	M3-2: 10
M8-1: 15	M2-1: 9	M6-4: 15.3	M11-2: 14	M17-2: 13	M12-1: 18, 19	M5-5: 14
MX-1: 26	M13-1: 15	M15-3: 20.3, 22.3				

Quality Control Testing

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

Storage and Handling

GLUTag 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

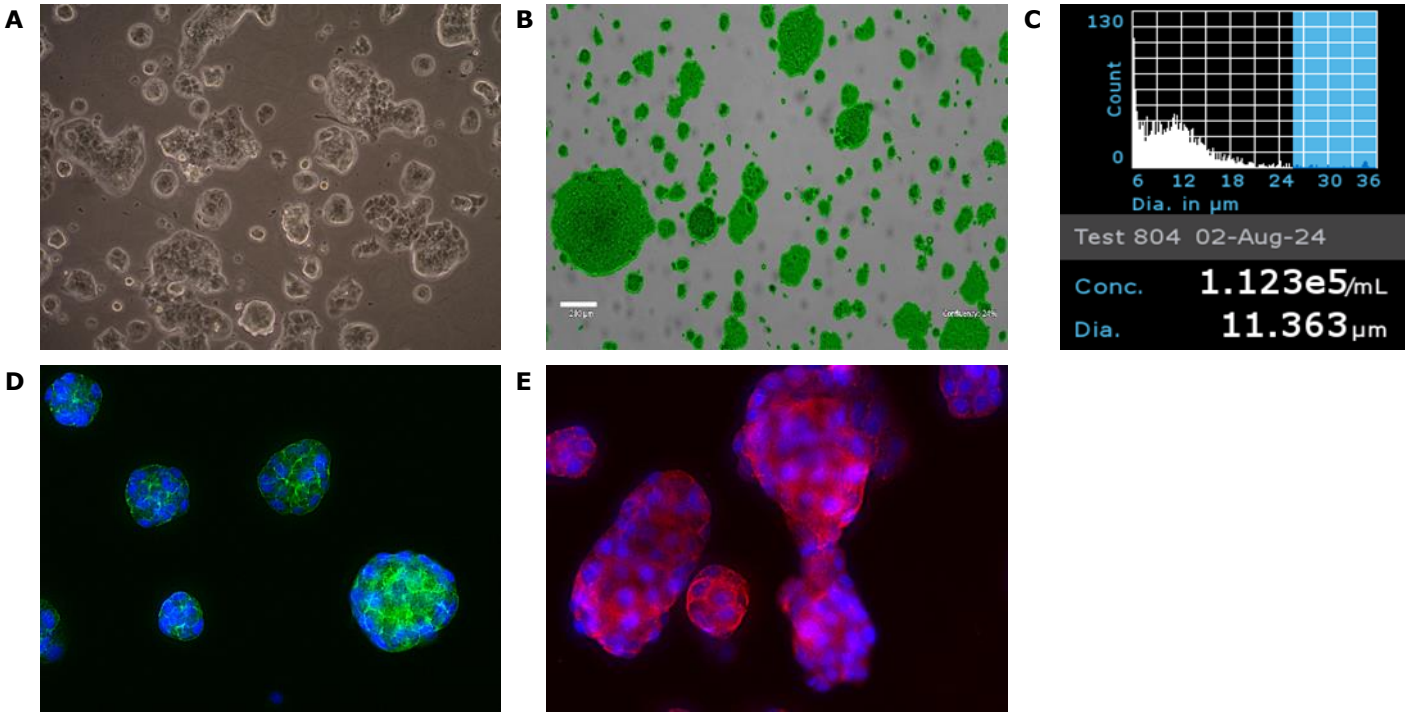

Figure 1. (**A**) Bright-field images of GLUTag cells three days after thaw in a T75 flask (4X magnification). (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using the Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensors (PHCC360KIT). (**D**) GLUTag cells stained with Phalloidin-Atto-488 (49409). (**E**) GLUTag cells express GLP-1 (Thermo, BS-0038R).

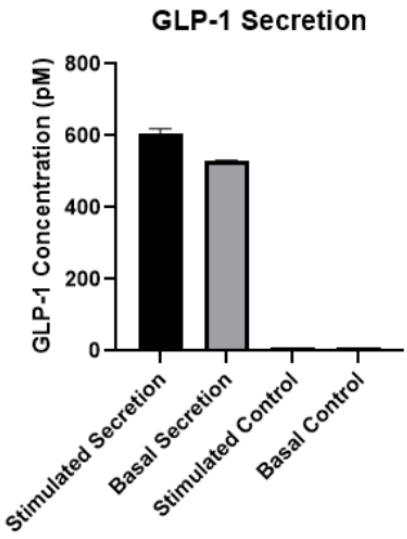

Figure 2. GLUTag cells secrete GLP-1 at a basal level in addition to an increased secretion when stimulated with forskolin and isobutyl-methylxanthine (IBMX). Stimulated samples were harvested from GLUTag culture supernatant media after 2 hours of stimulation with 10 µM Forskolin and 10 µM IBMX. Basal secretion samples were harvested from regular cell media supernatant (DMEM 10% FBS) from GLUTag culture which had been growing for 72 hours. Basal control contains only fresh DMEM with 10% FBS and stimulated control contains only fresh DMEM with 10% FBS, 10 µM forskolin, and 10 µM IBMX. Samples were tested via Interspecies GLP-1 ELISA Kit (EZGLP1T-36K).

Protocols

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.
2. GLUTag cells are thawed and expanded in GLUTag Expansion Medium comprising of DMEM (D5796) containing 10% FBS (ES-009-B), 2 mM L-Glutamine (G7513) and Penicillin/Streptomycin (P4333).
3. Remove the vial of frozen GLUTag 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.
4. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
5. 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.
6. Using a 10 mL pipette, slowly add dropwise 9 mL of GLUTag Expansion Medium (step 2 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.
7. 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.
8. Centrifuge the tube at 300 x *g* for 2-3 minutes to pellet the cells.
9. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
10. Resuspend the cells in 15 mL of GLUTag Expansion Medium.
11. Transfer the cell mixture to a T75 tissue culture flask.
12. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. GLUTag cells can be passaged at approximately 80-85% confluency.
2. Carefully remove the medium from the tissue culture flask containing the 80-85% confluent layer of GLUTag cells.
3. Rinse the flask with 10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 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 GLUTag 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 GLUTag cell 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 or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:10.

Cryopreservation of the Cells

GLUTag cells may be frozen in GLUTag Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Drucker DJ, Jin T, Sylvia L, Young TA, Brubaker PL. 1994. Activation of proglucagon gene transcription by protein kinase-A in a novel mouse enteroendocrine cell line. Molecular Endocrinology. 8(12):1646–1655. doi:https://doi.org/10.1210/mend.8.12.7535893.
2. Kuhre RE, Wewer Albrechtsen NJ, Deacon CF, Balk-Møller E, Rehfeld JF, Reimann F, Gribble FM, Holst JJ. 2016. Peptide production and secretion in GLUTag, NCI-H716, and STC-1 cells: a comparison to native L-cells. Journal of Molecular Endocrinology. 56(3):201–211. doi:https://doi.org/10.1530/jme-15-0293.

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