Data Sheet

GRINCH Rat Insulinoma Cell Line

SCC611

Pack Size: ≥ $1x10^6$

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

Approximately 1 in 11 adults globally has diabetes, leading to 12% of health expenditure being diabetes related.[1] Diabetes mellitus refers to a group of metabolic diseases involving chronic hyperglycemia due to reduced insulin secretion, insulin synthesis, or increased insulin resistance. Recent advancements in technology have enabled the molecular understanding of diabetes in childhood and non-obese patients, unveiling the role of single gene defects. These defects, found in genes encoding beta-cell components like potassium channels, glucokinase, transcription factors, and insulin, contribute to 1-5% of all diabetes cases and are characteristic of monogenic diabetes.[2] Insulin gene mutations can lead to various diabetes subtypes by affecting insulin folding, causing retention in the endoplasmic reticulum (ER), triggering ER stress, beta-cell death, and impairing insulin-insulin receptor (IR) interaction.

Glucose-Responsive Insulin-secreting C-peptide modified Human proinsulin (GRINCH) is a rat insulinoma cell line derived from the INS1 cell line. INS1 cells were transfected with the hPro-CpepSfGFP construct containing the human insulin with a C-peptide modified with a C-terminal super folder GFP insert.[3] The GRINCH cell line exhibits efficient glucose-stimulated insulin secretions, making them a good model to study insulin secretion regulation and pancreatic β-cell function.[3] The fluorescent C-peptide is co-stored and co-secreted with human insulin allowing the tracking and quantification of insulin biosynthesis in real time.

Source

The GRINCH cell line was derived from INS1 cells. The INS1 cell line was derived from X-Ray induced insulinoma in rats.

Quality Control Testing

The GRINCH cell line is verified to be of rat origin and negative for mouse, human, Chinese hamster, Golden Syrian hamster, 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 against a Mouse/Rat Essential CLEAR panel by Charles River Animal Diagnostic Services.

Cells tested negative for mycoplasma.

Storage and Handling

The GRINCH 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

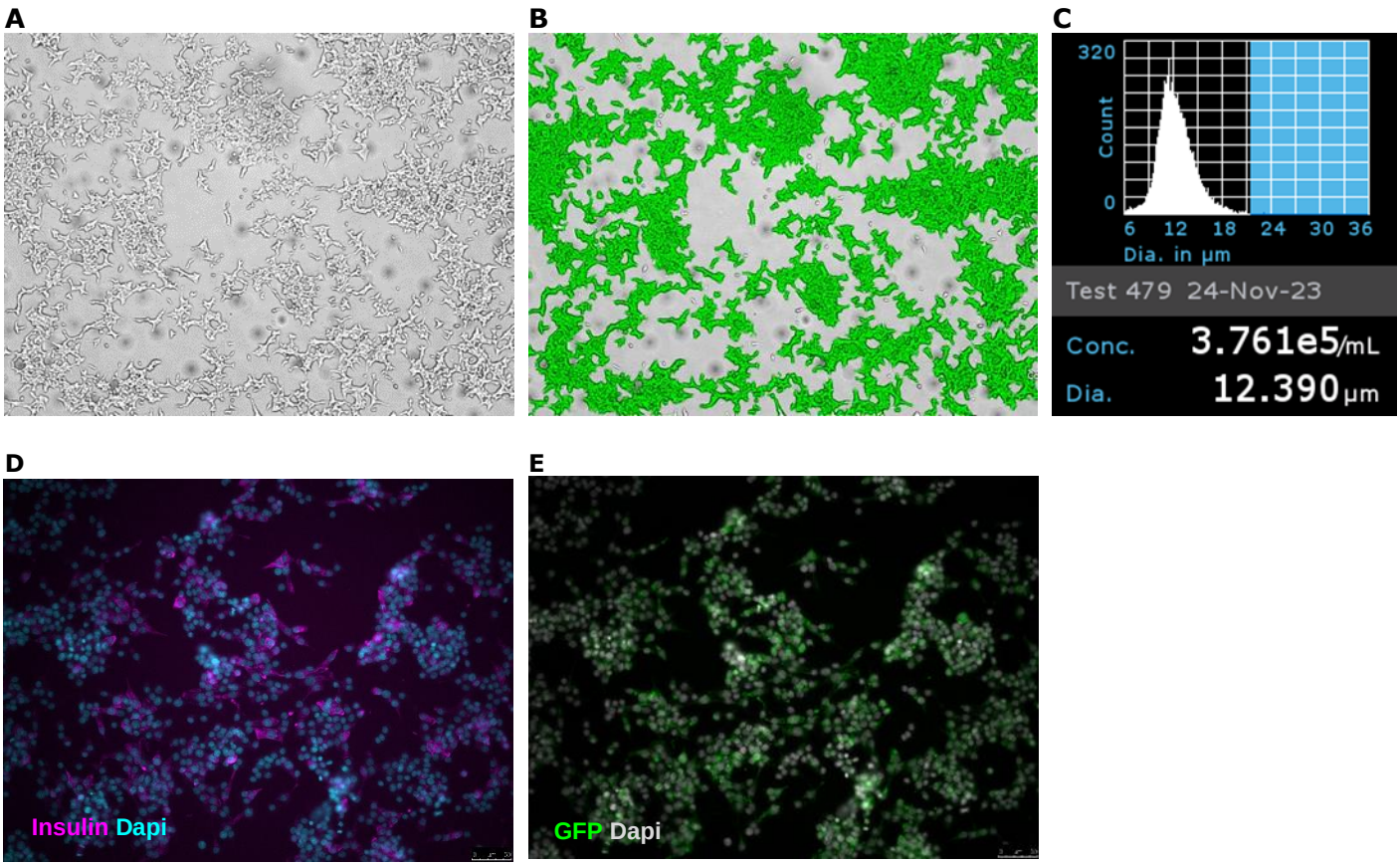

Figure 1. (**A**) Bright-field image of GRINCH cells. Cell confluency was assessed throughout the culture using the MilliCell® Digital Cell Imager. (**B**) Confluency at 49% is shown (MDCI10000). (**C**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT). (**D**) Cells express Insulin (I2018), and (**E**) GFP.

Protocols

Please refer to the protocol for thawing, subculturing and cryopreservation before starting the culture. The GRINCH cell line requires passaging before it reaches 60% confluency and cryopreservation using CryoStor® Cell Cryopreservation Media to ensure adequate viability at recovery. The GRINCH cell line requires 2-mercaptoethanol supplementation in the culture media.

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. Cells are thawed and expanded in GRINCH Expansion Medium comprising RPMI1640 medium (R0883) containing 10% FBS (for example, ES-009-B), 1 mM Sodium pyruvate (S8636), and EmbryoMax® 2-mercaptoethanol at 1X (ES-007-E) or 0.05 mM final concentration.
2. Remove the vial of frozen GRINCH 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 GRINCH 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 GRINCH Expansion Medium and transfer the cell mixture to a T75 tissue culture flask.
10. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. **Do not allow the cells to grow above 60% confluency**. The GRINCH cells should be passaged at 40-60% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 60% confluent layer of GRINCH cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 3-5 mL of Accutase® 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 GRINCH 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 GRINCH 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 or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

The GRINCH cells must be frozen in CryoStor® Cell Cryopreservation Media (C2874) using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Trikkalinou A, Papazafiropoulou AK, Melidonis A. 2017. Type 2 diabetes and quality of life. World Journal of Diabetes. 8(4):120.
2. Yang Y, Chan L. 2016. Monogenic Diabetes: What It Teaches Us on the Common Forms of Type 1 and Type 2 Diabetes. Endocrine Reviews. 37(3):190–222.
3. Haataja L, Snapp EL, Wright JJ, Li M, Hardy A, Wheeler MB, Markwardt ML, Rizzo MA, Arvan P. 2013. Proinsulin Intermolecular Interactions during Secretory Trafficking in Pancreatic β Cells. 288(3):1896–1906.

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