Data Sheet

INS-1E Rat Insulinoma Beta Cell Line

Cloned Cell Line

SCC491

Pack Size ≥ 1x10^6 cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

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

Background

Diabetes is a disease characterized by prolonged high blood glucose levels due to low production of insulin by the pancreas, or by cells no longer properly responding to insulin. Failure of islet beta cells of the pancreas in Type 2 diabetes occurs when islets are unable to maintain β cell compensation for insulin resistance. The failure is progressive, which leads to dysfunctional, de-differentiated β cells and loss of β cell mass secondary to apoptosis.

Beta-cell models are therefore essential tools for the study of diabetes. In many cases, the use of primary cells is preferred—but *in vitro* studies require large quantities of cells, which may make the use of primary cells more challenging, and primary cells typically also inconveniently contain mixed populations of cells. A variety of rodent-based beta-cell models are available, but many of these models are less than ideal due to improper response to glucose, poor differentiation, and low insulin content.[1]

The INS-1E cell line is an isolated clone from the parental INS-1 pancreatic β-cell line. Cells that produced high insulin content as well as a proper secretory response to glucose were isolated to become the INS-1E cell line. This cell line has shown high stability and proper secretory responses even after a high number of passages.[1] This presents a notable improvement in beta-cell models due to its capability for beta-cell differentiation and stability over many passages.

Source

INS-1E cell line was produced by cloning parental INS-1 cells. INS-1E cells were selected from the parental cells based on insulin content and secretory response to glucose. The parental INS-1 cell line pancreatic beta islet cell line was isolated from rat.

Quality Control Testing

- INS-1E cells are 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 Comprehensive CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

INS-1E 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

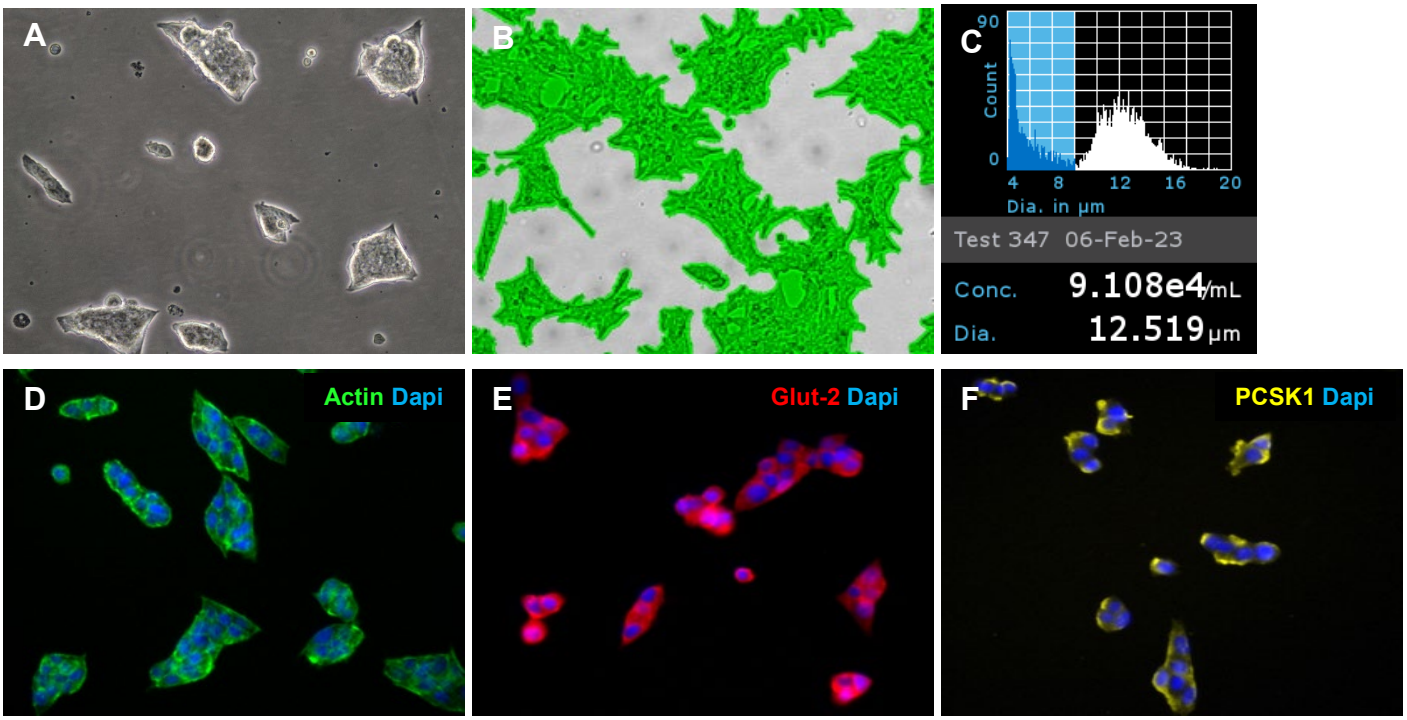

Figure 1. A. Bright-field image of INS-1E cells two days after thaw in a T75 flask. **B.** Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). **C.** Cell counting was performed using the Scepter™ 3.0 handheld automated cell counter using 40 µm sensors (PHCC340KIT). **D.** Cells express actin (49409), **E.** Glut-2 (SAB5701026), and **F.** PCSK1 (WH0005122M2).

Protocols

Thawing the Cells

Cells should be thawed in a T25 flask. Higher cell density is recommended for thaw. Product catalog numbers are indicated in ( ) and can be purchased at SigmaAldrich.com unless otherwise stated.

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 **INS-1E Expansion Medium** comprising RPMI-1640 medium (R8758) containing 10% FBS (e.g. ES-009-B), 2 mM L-Glutamine (G7513), 10mM HEPES (TMS-003-C), 1 mM Sodium Pyruvate (TMS-005-C), and 50 µM 2-Mercaptoethanol (M6250).
2. Remove the vial of frozen INS-1E 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 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 INS-1E Expansion Medium.
10. Transfer the cell mixture to a T25 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

To preserve differentiation potential, cells should be passaged once a week and plated at a seeding density of 40,000 cells/cm^2. Do not allow the cells to grow to confluency.

1. Carefully remove the medium from the T25 tissue culture flask containing the INS-1E cells.
2. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 3-5 mL of Accutase® and incubate in a 37 °C incubator for 3-5 minutes.
4. Inspect the flask and gently tap the side of the flask with the palm of your hand. Do not over-trypsinize. Cells favor clumping. Stop cell detachment as soon as you see cells lifting off the plate.
5. Add 5-7 mL of INS-1E 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 INS-1E Expansion Medium to the conical tube and lightly resuspend the cells. **IMPORTANT:** Do not vortex the cells.
10. Count the number of cells using a hemocytometer or a Scepter™ 3.0 handheld automated cell counter.
11. Plate the cells at 40,000 cells/cm^2.

Cryopreservation of the Cells

INS-1E cells may be frozen in INS-1E Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Merglen A, Theander S, Rubi B, Chaffard G, Wollheim CB, Maechler P.2004. Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells. Endocrinology. 145(2):667-678.

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