INS-1 832/13 Rat Insulinoma Cell Line

Cancer Cell Line

Cat. # SCC207

Pack size: ≥1x10^6 viable cells/vial

Store in liquid nitrogen

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Data Sheet

Background

Glucose-stimulated insulin secretion (GSIS) is potentiated by pancreatic beta cells and is critical to the physiological control of blood glucose levels. Insulin secretion is impaired in type 2 diabetes, and insight into the mechanisms and regulation of insulin secretion is fundamental to understanding the roles of beta cells in metabolic disease.

The rat insulinoma cell line INS-1[1] is a well-established model for studies of pancreatic islet beta-cell function; however, the GSIS response of INS-1 cells may decrease over time. The INS-1 832/13 cell line is a subclone of INS-1 that was selected for robust GSIS, producing and secreting both rat and human insulin. INS-1 832/13 harbors a human insulin expression cassette allowing for human insulin secretion to be maintained over extended passages with selection[2]. INS-1 832/13 cells exhibit enhanced secretory responsiveness to glucose as compared with the parental INS-1 cell line[2]. INS-1 832/13 cells may be characterized by granular staining for synaptotagmin, as described for the parental cell line[3]. These characteristics make INS-1 832/13 cells a useful system for investigating mechanisms of cellular insulin secretion, storage and synthesis.

Source

INS-1 832/13 is a derivative of INS-1 cells originally established from an x-ray induced insulinoma in rat[1]. The INS-1 832/13 cell line is a subclone of INS-1 that was stably transfected with a CMV promoter-human insulin expression plasmid carrying a geneticin (G418)-resistance marker for selection[2].

Storage and Handling

INS-1 832/13 Rat Insulinoma Cell Line should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- Cells are tested negative for infectious diseases by a Mouse/Rat Comprehensive CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of rat origin and negative for inter-species contamination from mouse, chinese hamster, Golden Syrian hamster, human and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Representative Data

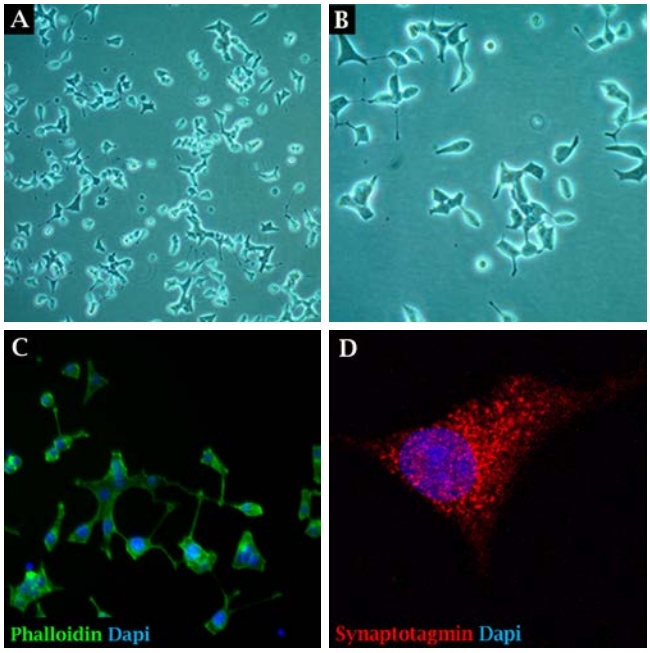

Figure 1. INS-1 832/13 cells one day after thawing in a T75 flask (A, 10X magnification), (B, 20X magnification). Cells express actin (Phalloidin, C) and Synaptotagmin (D, red).

References

1. Asfari M, Janjic D, Meda P, Li G, Halban PA, Wollheim CB (1992). Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines. *Endocrinology* 130(1): 167-178.
2. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB (2000). Isolation of INS-1-derived cell lines with robust ATP-sensitive K+ channel-dependent and –independent glucose-stimulated insulin secretion. *Diabetes* 49(3): 424-30.
3. Lang J, Fukuda M, Zhang H, Mikoshiba K, Wollheim CB. (1997). The first C2 domain of synaptotagmin is required forexocytosis of insulin from pancreatic beta-cells: action of synaptotagmin at low micromolar calcium. *EMBO J* 16(19):5837-5846.

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Protocols

Note: Extensive passaging may cause cells to lose the expression of the human insulin gene and the population may become heterogeneous for expression of human insulin. However, the loss of human insulin expression does not affect cell function measured as GSIS. G418 at 0.3 mg/mL may be added to apply selective pressure when growing larger batches of cells.

Thawing 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.

   INS-1 832/13 Expansion Medium: Cells are thawed and expanded in RPMI-1640 (Sigma Cat. No. R0883) supplemented with 2 mM L-Glutamine (Cat. No. TMS-002-C),1 mM sodium pyruvate (Cat. No. TMS-005-B), 10 mM HEPES (Cat. No. TMS-003-C), 0.05 mM β-mercaptoethanol (Cat. No. ES-007-E) and 10% FBS (Cat. No. ES-009-B).

   Note: β-mercaptoethanol is critical for the continued propagation of the cell line and should not be omitted from the culture medium.
2. Remove the vial of frozen INS-1 832/13 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 INS-1 832/13 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-1 832/13 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 Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of INS-1 832/13 cells.
2. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
3. Apply 5-7 mL of Accutase or trypsin-EDTA solution and incubate in a 37°C incubator for 3-5 minutes.
4. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
5. Add 5-7 mL of INS-1 832/13 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-1 832/13 Expansion Medium to the conical tube and resuspend the cells thoroughly.

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

Cryopreservation of Cells

INS-1 832/13 rat insulinoma cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

Glucose stimulated insulin secretion (GSIS):

For each condition, triplicate wells should be plated.

1. Cells are seeded at a density of $0.5x10^6$/well in 1 mL medium for a 24-well plate, or $1x10^6$/well in 2 mL of medium for a 12-well plate. For best results in glucose stimulated insulin secretion, cells need to be confluent.
2. After 2 days, the medium should be changed, and the assay should be performed on day 3.
3. The glucose stimulated insulin secretion is performed in HBSS (Hepes balanced salt solution): 114 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4,1.16 mmol/L $MgSO_4$, 20 mmol/L HEPES, 2.5 mmol/L $CaCl_2$, 25.5 mmol/L $NaHCO_3$, and 0.2% bovine serum albumin, pH 7.2. (Hohmeier et al. 2000, Diabetes 49:424-430).
4. For the assay, wash the cells twice with HBSS + 2.5 mM glucose. The first wash is just a quick rinse, for the second wash, leave the HBSS on for 1 hr.

   Caution: The cells do not attach firmly to the plates and will wash off very easy, if solutions are added with too much force.
5. After 1 hrs, the secretagogues diluted in HBSS are added for 2 hr. For a 24-well plate, add 1mL/well and 1.5 or 2 mL/well to a 12-well plate.
6. After 2 hrs, remove the solution for insulin ELISA. Keep in mind that these cells secrete a mixture of rat and human insulin and an insulin ELISA which is cross reactive between these two species will give you higher sensitivity – especially if you want to adapt the assay for a 96-well format. We recommend the Chemi-rodent-insulin ELISA from ALPCO Cat. No. 80-INSMR-CH01.
7. Wash the cells twice with PBS and lyse cells with RIPA buffer to measure total cellular protein for normalization of secreted insulin to protein.
8. Conditions used for quality control:

   1. HBSS+2.5 mM glucose (basal insulin secretion)
   2. HBSS+15 mM glucose (stimulated insulin secretion)
   3. HBSS+15 mM glucose +50 nM GLP-1

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