Cell Line Designation: INS-1 832/13

AddexBio Catalog No. C0018024

Cell Line Description:

Disease: Insulinoma

Origin: INS-1 832/13 is a derivative of INS-1 (C0018007) cells originally established from an x-ray induced insulinoma in rat. 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.

Species: Rattus Norvegicus

Tissue: Pancreas

Properties: Adherent; β cells

Recommended Culture Flask: BD Biosciences Tissue Culture-treated flask

Complete Medium: AddexBio Optimized RPMI-1640 (C0004-02) + 10% FBS + 0.05 mM β -mercaptoethanol (Gibco 21985023). **Note**: β-mercaptoethanol is critical for the continued propagation of the cell line and should not be omitted from the culture medium.

Selection: Extensive passaging may cause cells to lose the expression of the human insuline 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. Apply G418 one day after cells are attached to the flask/plate. Do not apply G418 during the day the cells are trypsinized and plated to the new flask, the line may be lost.

Subculture Procedure: Volumes are given for a 75 cm^2 flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while

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waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: 1:5 to 1:6 is recommended

Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 7.5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafty/bmbl4/bmbl4toc.htm

Use Restrictions: **These cells are distributed for research purposes only.** Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Cells Upon Arrival:

Frozen cells must be thawed immediately upon receipt and grown according to the handling procedures described here in this instruction manual to ensure the best cell viability.

*Note: **Avoid refreezing or repetitive freezing cells upon receipt** as it may result in irreversible damage to the cell line.*

Disclaimer: We cannot guarantee cell viability if the cells are not thawed immediately upon receipt and grown according to handling procedures described in this instruction manual.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be

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stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.

3. Resuspend cell pellet with the recommended complete medium and dispense into a new culture flask (T25). It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

4. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics), as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.
2. **For the cells are still attached**, asceptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **For the portion of cells that are not attached**, asceptically remove the entire contents of the flask but 10 ml of the shipping medium and centrifuge at 125 x g for 5 minutes.

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Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to the same 25 cm^2 flask (T25). Incubate at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

4. Cells will need some time to recover from the shipping journey. Keep watching the cells and replace medium (10 ml) every two days without disturbing the monolayer for the first week or until they are 80-85% confluent.

References for INS-1 832/13 cells:

1. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robutst ATP-sensitive K+ channel-dependent and -independent glucose-stimulated insulin secretion. Diabetes, 2000;49(3):424-430. PMID: 10868964

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Lot Specific Information Sheet for AddexBio Cat #: C0018024

Lot Number: 0034879

Designation: INS-1 832/13 CELLS

Total Cells/mL: >1.2x10^6

Expected Viability: 70.0-75.0%

Ampule Passage #: 12

Dilute Ampule Content: 1:10 (T-25) or 1:15 (T-75)

Volume/Ampule: 1 mL

A T-25 setup at a dilution of 1:10, using culture medium as described in the product information sheet, reaches approximately 40-45% confluence within 24 to 48 hours.

Remarks to INS-1 832/13 cell line (Please read before you proceed):

This cell line is not attaching well at the beginning when you thaw the cells or after trypsinized. Therefore, please allow more time to let them attach to the flask (usually 24-48 hours without changing the medium). It is normal to observe floating cells the first few days after thawing. This cell line grow slowly at first. There are times cells may form attached clumps and spread slowly after thawing. After 3 days of thawing, start feeding the cells until they are become more confluent and more established before subculture.

Please make sure that mercaptoethanol at the specified concentration are added to the medium for the cells to grow. Missing any of these components will result in failure to attach and grow.

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