Cell Line Designation: MIN-6

AddexBio Catalog No. C0018008

Cell Line Description:

Disease: Insulinoma (SV40 T-antigen induced cell immortalization)

Origin: Pancreatic Islets

Species: Mus musculus

Tissue: Pancreas

Properties: Adherent; β cells

Recommended Culture Flask: BD Biosciences Tissue Culture-treated flask

Complete Medium: AddexBio Advanced Medium (C0003-04) + 15% FBS + 0.05 mM 2-mercaptoethanol (Invitrogen Cat: 21985023). **Please note:** *in order to allow successful cell attachment to the flask, use ONLY new batch of Advanced Medium and other components. Other components should be added freshly to make the complete medium before use. Complete medium stored in the refrigerator for an extended period of time (> 1-2 weeks) may lose efficacy to maintain cell attachment and cell growth, and therefore, it should be discarded.*

Subculture Procedure: 1:2 to 1:3 (80% confluency) using 0.25% trypsin or trypsin/EDTA, 5% CO_2; 37°C

Medium Renewal: Two to three times weekly.

Freezing Medium: Culture medium supplemented with 20% (v/v) FBS and 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: II

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical

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AddexBio Technologies
4907 Morena Blvd, Ste 1408
San Diego, CA 92117 USA

858-348-7819
Fax: 858-538-8847
Email:customersupport@addexbio.com
www.addexbio.com

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 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. Transfer the vial content to a sterile T25 flask containing 9.0 mL complete culture medium.

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4. 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).

5. Incubate the culture at 37°C in a suitable humidified incubator. A 5% CO_2 in air atmosphere is recommended. It is recommended to allow the cells to grow without disturbing them for 2-3 days. Try to avoid shaking of the flask when handling.

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. 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 70-80% confluent.

References for MIN-6 cells:

1. Ishihara, H., Asano, T., Tsukuda, K. et al. (1993). Pancreatic beta cell line MIN6 exhibits characteristics of glucose metabolism and glucose-stimulated insulin secretion similar to those of normal islets. Diabetologia 36(11), 1139-1145.

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Lot Specific Information Sheet for AddexBio Cat #: C0018008

Lot Number: 0010138

Designation: MIN-6 CELLS

Total Cells/mL: >$2x10^6$

Expected Viability: 49.2%

Ampule Passage #: 11

Dilute Ampule Content: 1:10 (T-25)

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 60-70% confluence within 24 to 48 hours.

Important Remarks to MIN-6 cell line (Please read before you proceed):

Please use our formulated medium or use medium with comparable formulation. This cell line is not attaching well at the beginning when you thaw the cells or after trypsinization. Therefore, please allow more time to let them attach to the flask (2-3 days). It is normal to observe floating cells the first few days after thawing. This cell line grow slowly. These cells are fragile, use care when adding medium or handling flask. Do not change medium the first 2-3 days after thawing or splitting. Do not flush the cells directly with medium. The cells should not be allowed to become confluent, subculture at 80% of confluence.

Using ONLY AddexBio Advanced Medium (C0003-04) is strongly recommended as MIN-6 cells are very sensitive to some components in the medium for attachment. Using media (from other vendors) that do not contain the same formulation could lead to poor cell performance and will void warranty.

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