Code:	0293
Cell Line:	MIN-6
Species:	Mus musculus **Vulgar Name:** Mouse
Tissue:	Pancreas
Disease:	Tumor
Growth Properties:	Adherent
Products:	MIN6 cells excreted insulin, glucagon, somatostatin and ghrelin.
Biosafey:	2
Additional info:	MIN6 cells excreted insulin, glucagon, somatostatin and ghrelin.
Culture Medium:	RPMI-1640 medium modified to contain 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, 4500 mg/L glucose, and 1500 mg/L sodium bicarbonate, 10% fetal bovine serum and 0.05 mM de 2-mercaptoethanol

Subculturing:	Subculture when 80% confluent or less. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which contains trypsin inhibitor. 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). Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension into new culture vessels. Incubate cultures at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010. **Medium Renewal:** 2 to 3 times per week **Subcultivation ratio:**
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5%Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:

SAFETY PRECAUTION: Is highly recommend 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 Oring and cap out of the 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. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.

4.Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio).

5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line).

NOTE: 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 growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

References:

Garay-Malpartida et al.: Toll-like receptor 4 (TLR4) expression in human and murine pancreatic beta-cells affects cell viability and insulin homeostasis. BMC Immunology 2011 12:18;

Helen Roderigo-Milne et al: Differential expression of insulin genes 1 and 2 in MIN6 cells and pseudoislets. Biochemical and Biophysical Research Communications 296 (2002) 589–595.

Nakashima k, MIN6 is not a pure beta cell line but a mixed cell line with other pancreatic endocrine hormones. Endocr J. 2009;56(1):45-53, 2008. PubMed: 18845907

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