Data Sheet

MIN6 Mouse Insulinoma Cell Line

SCC623

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

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

Background

The MIN6 Mouse Insulinoma Cell Line, was derived from an insulinoma tissue that developed in a transgenic mouse in which the expression of the Simian Virus 40 Large T antigen was driven by the human insulin promoter.[1,2,3] In comparison to the primary pancreatic β-cells, MIN6 Mouse Insulinoma Cell Line thrives reliably and far more robustly in cell culture and yet retains the important characteristics of the β-cells, such as the exclusive expression of the so-called "liver-type" glucose transporter (Glut2) and their ability to modulate the insulin secretion in response to the changing glucose concentration in the growth medium, and it has been a staple strain in diabetes research since its establishment in 1990.[1]

Additionally, the expression of the major histocompatibility complex I (MHC I) is lacking in MIN6, as in the non-diabetic β-cells, but can be induced by pro-inflammatory cytokines,[1,4,5] which would render them susceptible to T-cell mediated cytotoxicity. This closely mimics the behavior of the β-cells isolated from diabetic tissues, where chronic inflammation is commonly observed, and it has made MIN6 an attractive cell line model for investigating the mechanism of the β-cells loss due to their autoimmune destruction.

Source

The MIN6 Mouse Insulinoma Cell Line was derived from a pancreatic tumor arising in a transgenic mouse created by the microinjection of the SV40 large-T antigen fused with the human insulin promoter into the pronuclei of fertilized C57BL/6 embryo and cultured after a single cloning step.[1,2,3]

Short Tandem Repeat

M18-3:	16	M1-2:	19	M8-1:	16	M11-2:	16	MX-1:	28
M4-2:	20.3	M7-1:	26, 26.2, 27.2	M2-1:	16	M17-2:	15	M13-1:	16, 17
M6-7:	15, 17	M1-1:	16, 17	M15-3:	22.3	M12-1:	17	M19-2:	13
M3-2:	14	M6-4:	18	M5-5:	17				

Quality Control Testing

- The MIN6 Mouse Insulinoma Cell Line is verified to be of mouse origin and negative for human, rat, 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 Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The MIN6 Mouse Insulinoma 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

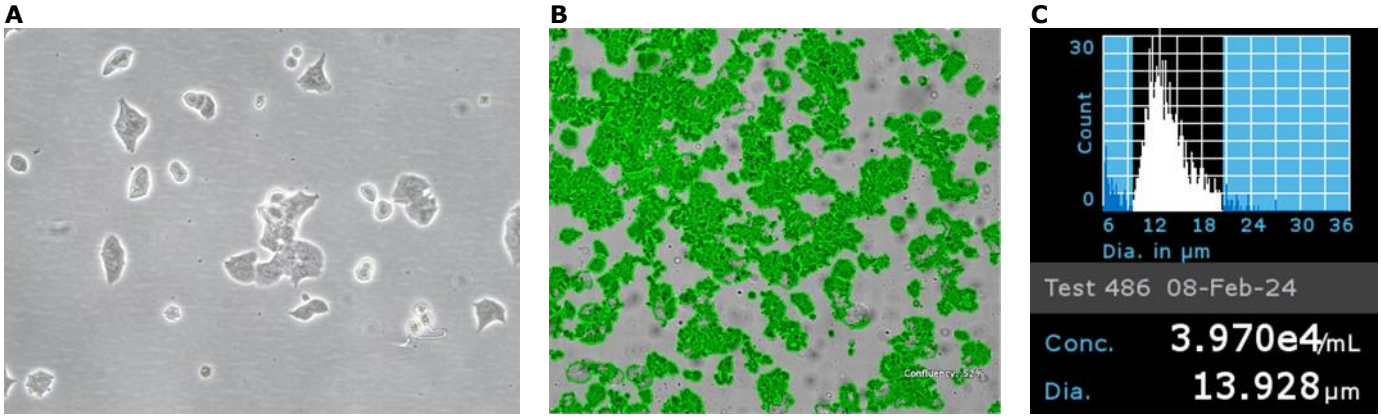

Figure 1. (**A**) Bright-field image of MIN6 Mouse Insulinoma cells a day after thaw in a T75 flask. (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT).

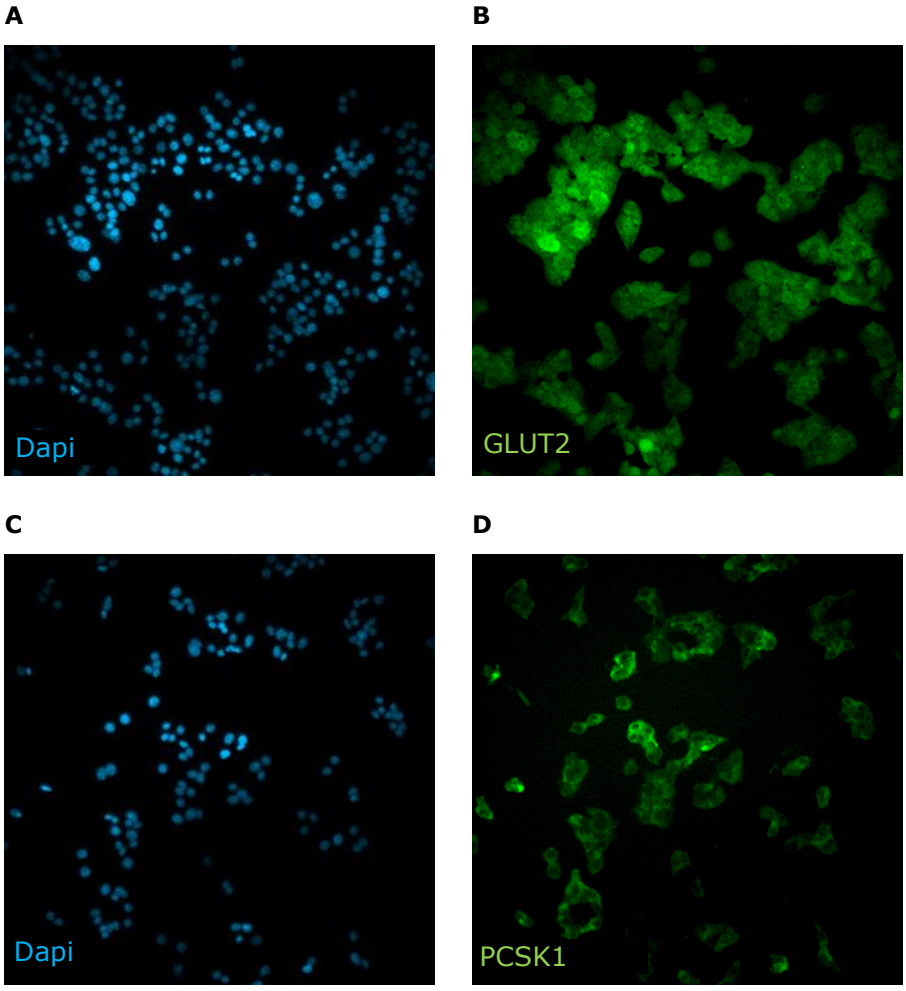

Figure 2. MIN6 Mouse Insulinoma cells express the pancreatic β-cell markers. (**A** and **C**) MIN6 Mouse Insulinoma cells labeled with DAPI (MBD0015), (**B**) anti-Glut2 antibodies (SAB5701026), and (**D**) anti-PCSK1 antibodies (WH0005122M2).

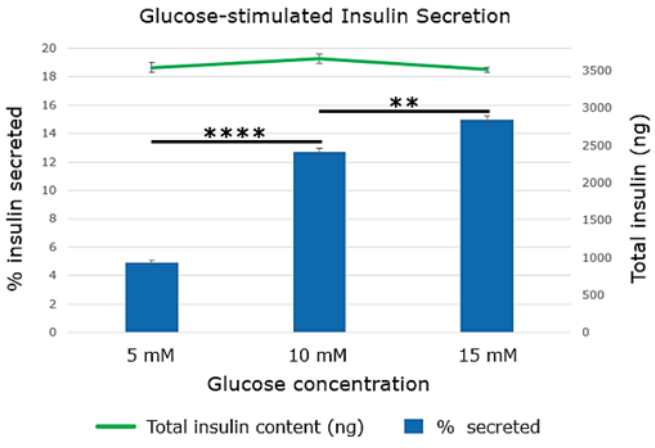

Figure 3. Glucose-stimulated insulin secretion by MIN6. Approximately 350,000 MIN6 Mouse Insulinoma cells were grown in the expansion media for 48 hours, before 30 minutes of glucose starvation and stimulation with the indicated concentration of glucose for 30 minutes. The amount of insulin secreted into the media increased as the glucose concentration rose, while little difference was observed in the total insulin content of the culture. The Rat/Mouse Insulin 96 well Plate Assay kit (EZRMI-13K) was used for ELISA. Bars represent N=3 +/- standard deviation. The p-values for the two-tailed t-tests were 0.00004984 for ****and 0.0050132 for **.

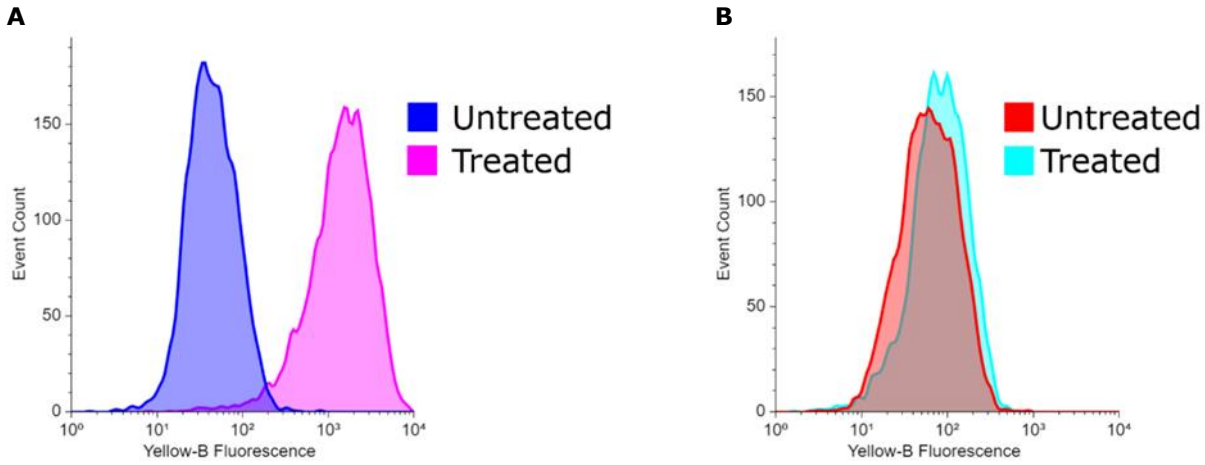

Figure 4. (**A**) Induced expression of the major histocompatibility complex I (MHC-I) has been observed in MIN6 in response to inflammatory cytokines[1,4,5], including INFγ. Cells were treated with 10 ng/mL INFγ for 72 hours, stained with anti-mouse MHC-I (34-1-2S)-PE antibody (Invitrogen, 12-5998-81), and analyzed by Guava® EasyCyte HT Flow Cytometer. (**B**) No comparable increase in fluorescence signal was observed using an isotype control (Invitrogen, 12-4724-81).

Protocols

Thawing the Cells

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. The MIN6 Mouse Insulinoma cells are thawed and expanded in MIN6 Expansion Medium comprising of DMEM (DF-042-B) containing 15% FBS (ES-009-B, heat-inactivated at 57 °C for 30 minutes) and β-mercaptoethanol (ES-007-E) with optional Penicillin/Streptomycin (P4333).
2. Remove the vial of frozen 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 MIN6 Expansion Medium (medium composition in Step 1) to the 15 mL conical tube.

   Important: The expansion medium should be pre-warmed to 37 °C. 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 150 x *g* for 3 minutes to pellet the cells.

   Important: Faster spin speed and/or longer centrifugation may result in decreased viability.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (CryoStor® Cell Cryopreservation Media, C2874).
9. Resuspend the cells in 15 mL of MIN6 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 the Cells

1. The MIN6 Mouse Insulinoma cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80-85% confluent layer of cells.
3. Rinse the flask with 7-10 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse. Repeat this wash step one more time.
4. Apply 2 mL of pre-warmed Accutase® (A6964) and incubate in a 37 °C incubator for 3-4 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 4-5 mL of MIN6 Expansion Medium to the flask.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 mL conical tube.
8. Centrifuge the tube at 150 x *g* for 3 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of MIN6 expansion medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    Important: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter™ 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Recommended density is 3-3.5 million cells for T75 flasks, and 6-7 million cells for T175 flasks.

Cryopreservation of the Cells

The MIN6 Mouse Insulinoma cells may be frozen in CryoStor® Cell Cryopreservation Media (C2874) using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Miyazaki J, Araki K, Yamato E, Ikegami H, Asano T, Shibasaki Y, Oka Y, Yamamura K. 1990. Establishment of a pancreatic beta cell line that retains glucose-inducible insulin secretion: special reference to expression of glucose transporter isoforms. Endocrinology. 27(1):126-32.
2. Sarvetnick N, Liggitt D, Pitts SL, Hansen SE, Stewart TA. 1988. Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. Cell. 52(5):773-82. Pytynia KB, Dahlstrom KR, Sturgis EM. 2014. Epidemiology of HPV-associated oropharyngeal cancer. Oral Oncol. 50(5): 380-6.
3. Ullrich A, Dull TJ, Gray A, Philips JA 3rd, Peter S. 1982. Variation in the sequence and modification state of the human insulin gene flanking regions. Nucleic Acids Res. 10(7):2225-40.
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5. Javeed N, Her TK, Brown MR, Vanderboom P, Rakshit K, Egan AM, Vella A, Lanza I, Matveyenko AV. 2021. Pro-inflammatory β cell small extracellular vesicles induce β cell failure through activation of the CXCL10/CXCR3 axis in diabetes. Cell Rep. 36(8):109613.

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