Data Sheet

IngWAT Mouse Immortalized Preadipocyte Cell Line

Immortalized Cell Line

SCC211

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 growing worldwide obesity epidemic has stimulated research into novel strategies to control and treat obesity and associated metabolic disorders. Great interest has been generated in brown fat, a specialized adipose tissue having increased energy expenditure, because manipulation of this tissue has potential to protect against diet-induced obesity by altering the balance of energy intake and consumption.[1] In common with brown fat, beige adipocytes possess thermogenic mechanisms, but have the unique property of inducible differentiation. Beige adipocyte differentiation occurs within white adipose tissue in response to environmental cues and is characterized by cristae-dense mitochondrial biogenesis and increased expression of uncoupling protein 1 (UCP1).[2]

The IngWAT mouse immortalized preadipocyte cell line is derived from subcutaneous white adipose tissue (WAT) isolated from a wild-type mouse.[2] IngWAT cells have been shown to support the induction of beige adipocytes and express UCP1.[2] IngWAT mouse immortalized preadipocyte cells are a versatile model for a broad spectrum of studies of beige adipocyte induction, maintenance and physiology.

Source

Stromal vascular fractions were isolated from the inguinal white adipose tissue of a wild-type mouse and immortalized by infection with Simian virus 40 (SV40) large T antigen.[2]

Quality Control Testing

- Each vial contains ≥ 1X10^6 viable cells.
- Cells are tested negative for infectious diseases by a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of mouse origin and negative for inter-species contamination from rat, 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.

Storage and Handling

IngWAT Mouse Immortalized Preadipocyte 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.

Representative Data

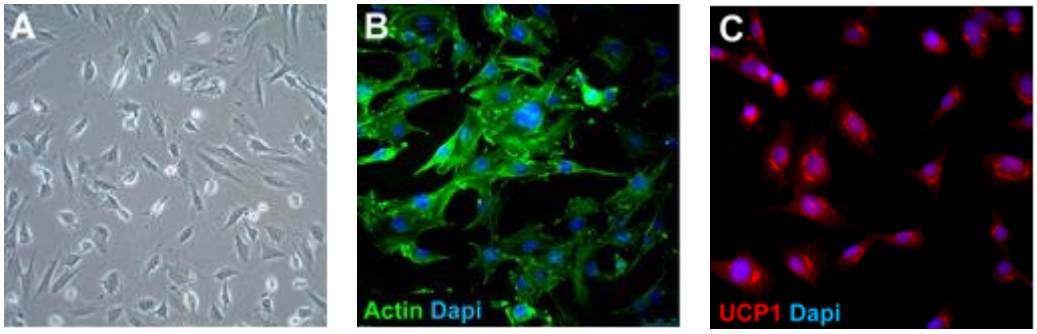

Figure 1. (**A**) IngWAT cells one day after thawing in a T75 flask. (**B**) Cells express actin and (**C**) UCPI.

Protocols

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.

   IngWAT Expansion Medium: Cells are thawed and expanded in DMEM/F12 PLUS basal medium (SCM162) supplemented with 10% FBS (ES-009-B).
2. Remove the vial of frozen IngWAT 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 IngWAT 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 IngWAT 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

Passage when cells are at 80% confluency. Cells proliferate rapidly. Do not allow cells to grow to 100% confluency.

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of IngWAT 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 IngWAT 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 IngWAT 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

IngWAT Mouse Immortalized Preadipocyte Cell Line may be frozen in the expansion medium plus 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Cui XB, Chen SY (2016) White adipose tissue browning and obesity. 31(1): 1-2.
2. Lu X et al. (2018) Mitophagy controls beige adipocyte maintenance through a Parkin-dependent and UCP1-independent mechanism. Sci Signal 11(527): eaap8526.

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