WT-1 Mouse Brown Preadipocyte Cell Line

Immortalized Cell Line

Cat. # SCC255

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.
THIS PRODUCT CONTAINS GENETICALLY MODIFIED ORGANISMS.

Data Sheet

Background

Obesity is a growing worldwide health epidemic that has spurred research into new avenues for treatment and control of metabolic disorders. Brown fat, a specialized adipose tissue characterized by increased energy expenditure, has emerged as an area of interest because manipulation of this tissue has potential to protect against diet-induced obesity by altering the balance of energy intake and consumption.[1]

The WT-1 mouse immortalized brown preadipocyte cell line is derived from primary brown preadipocytes. WT-1 preadipocytes can be induced to differentiate into brown adipocytes upon treatment with induction and differentiation factors. WT-1 cells express the brown fat differentiation factor BMP7 and brown fat marker uncoupling protein-1 (UCP1)[3] and are a valuable model for investigation into mitochondrial physiology during adipogenesis. The WT-1 cell line has been used to develop 3D assays for adipose tissue[4] and is well suited for a range of questions in cellular metabolic research.

Source

WT-1 cell line was established from primary brown pre-adipocytes isolated from newborn mouse pups and immortalized with Simian virus 40 (SV40) large T antigen.[2]

Storage & Handling

WT-1 mouse brown pre-adipocyte 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

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, human, chinese hamster, Golden Syrian hamster, and non-human primate (NHP) as assessed by a Contamination CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Representative Data

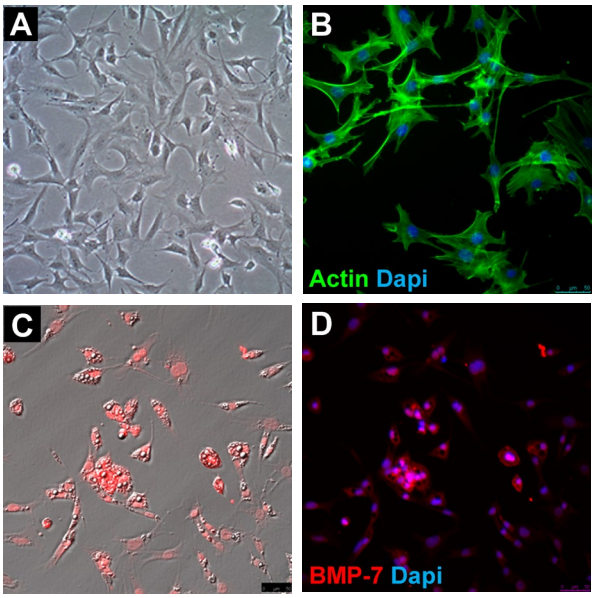

Figure 1. WT-1 cells one day (**A**) after thawing in a T75 flask. Cells express actin (**B**, Phalloidin-FITC; Sigma P5282). After 4 days of differentiation, cells accumulate lipid droplets (**C**) and express BMP-7 (**C, D**). BMP-7 is expressed by differentiating brown adipocytes. Figure C is a bright-field image overlaid with BMP-7 staining.

References

1. *J Biomed Res.* 2016 31(1): 1-2.
2. *PLoS One.* 2009 4(12): e8458.
3. *Nature.* 2008 454(7207): 1000-4.
4. *Biomaterials.* 2016 75: 123-134.

Rev.1.0/2020-01-14/SCC255/VC

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.

   WT-1 Expansion Medium: Cells are thawed and expanded in DMEM-High Glucose (Sigma Cat. No. D6429) supplemented with 10% FBS (Cat. No. ES-009-B).
2. Remove the vial of frozen WT-1 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 WT-1 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 WT-1 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

1. Do not allow the cells to grow to confluency. WT-1 cells should be passaged at ~80-85% confluence.
2. Carefully remove the medium from the T75 tissue culture flask containing the WT-1 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase and incubate in a 37°C incubator for 3-5 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 5-7 mL of WT-1 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
8. Centrifuge the tube at 300 x g for 3-5 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 WT-1Expansion Medium to the conical tube and resuspend the cells thoroughly.

    IMPORTANT: Do not vortex the cells.
11. Count the number of cells using a hemocytometer.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Differentiation Protocol

1. Prepare Induction Medium (50 mL) fresh on the day of use. Sterile filter.

Induction Media

		Cat. No.	Final Conc.	Notes
44 mL	DMEM High Glucose	D6429	89%	
5 mL	FBS	ES-009-B	10%	May be lowered to 2% if cells detach
0.6 µL	Insulin	I0516-5ML	20 nM	10 mg/mL stock or 1.7 mM
5 µL	Triiodo-L-Thyronine (T3)	T6397	1 nM	10 uM in methanol
50 µL	Indomethacin	I7378	0.125 mM	0.125M stock in methanol
50 µL	Dexamethasone	D2915	5 uM	5 mM stock or 2 mg/mL in water
500 µL	IBMX	I5879	0.5 mM	50 mM stock in 0.1M KOH
0.5 mL	Penicillin/Streptomycin	TMS-AB2	1X	
50 mL	**Total Volume**			

2. Prepare Differentiation Media. Sterile filter.

Differentiation Medium

		Cat. No.	Final Conc.	Notes
44.5 mL	DMEM High Glucose	D6429	89%	
5 mL	FBS	ES-009-B	10%	May be lowered to 2% if cells detach
0.6 µL	Insulin	I0516-5ML	20 nM	10 mg/mL stock or 1.7 mM
5 µL	Triiodo-L-Thyronine (T3)	T6397	1 nM	10 uM in methanol
0.5 mL	Penicillin/Streptomycin	TMS-AB2	1 X	
50 mL	**Total Volume**			

3. Day 0 – 2 (48 hours): Change to Induction Media

4. Days 3 – 8: Change to Differentiation media. Add fresh media every other day. Make sure to refresh media on the day BEFORE harvest for RNA, Oil Red O staining, protein etc.

Note: If differentiation is very high, cells may be ready for harvest on day 7 already b/c cells tend to detach and explode as culture gets older and more mature.

Cryopreservation of Cells

WT-1 mouse immortalized brown preadipocyte cell line may be frozen in the expansion medium plus 10% DMSO using a Nalgene slow freeze Mr. Frosty container.

ACADEMIC USE AGREEMENT
(subject to local law)

THIS PRODUCT MAY ONLY BE USED BY INDIVIDUALS EMPLOYED BY AN ACADEMIC INSTITUTION AND IS INTENDED SOLELY TO BE USED FOR ACADEMIC RESEARCH, WHICH IS FURTHER DEFINED BELOW. BY OPENING THIS PRODUCT, YOU ("PURCHASER") HEREBY REPRESENT THAT YOU HAVE THE RIGHT AND AUTHORITY TO LEGALLY BIND YOURSELF AND/OR YOUR EMPLOYER INSTITUTION, AS APPLICABLE, AND CONSENT TO BE LEGALLY BOUND BY THE TERMS OF THIS ACADEMIC USE AGREEMENT. IF YOU DO NOT AGREE TO COMPLY WITH THESE TERMS, YOU MAY NOT OPEN OR USE THE PRODUCT AND YOU MUST CALL MILLIPORESIGMA ("SELLER") CUSTOMER SERVICE (1-800-645-5476) TO ARRANGE TO RETURN THE PRODUCT FOR A REFUND.

"Product" means WT-1 Mouse Brown Preadipocyte Cell Line (SCC255)

"Academic Research" means any internal *in vitro* research use by individuals employed by an academic institution. Academic Research specifically excludes the following uses of whatever kind or nature:

- Re-engineering or copying the Product
- Making derivatives, modifications, or functional equivalents of the Product
- Obtaining patents or other intellectual property rights claiming use of the Product
- Using the Product in the development, testing, or manufacture of a Commercial Product
- Using the Product as a component of a Commercial Product
- Reselling or licensing the Product
- Using the Product in clinical or therapeutic applications including producing materials for clinical trials
- Administering the Product to humans
- Using the Product in collaboration with a commercial or non-academic entity

"Commercial Product" means any product intended for: (i) current or future sale; (ii) use in a fee-for-service; or (iii) any diagnostic, clinical, or therapeutic use.

Access to the Product is limited solely to those officers, employees, and students of PURCHASER's academic institution who need access to the Product to perform Academic Research. PURCHASER shall comply with all applicable laws in its use and handling of the Product and shall keep it under reasonably safe and secure conditions to prevent unauthorized use or access.

These use restrictions will remain in effect for as long as PURCHASER possesses the Product.

COMMERCIAL OR NON-ACADEMIC ENTITIES INTERESTED IN PURCHASING OR USING THE PRODUCT MUST CONTACT licensing@emdmillipore.com AND AGREE TO SEPARATE TERMS OF USE PRIOR TO USE OR PURCHASE.

GMO
This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient des organismes génétiquement modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.