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Technical Data Sheet: hTERT-immortalized Brown Preadipocytes

ATCC® Number	CRL-4062™
Organism	*Homo sapiens*
Tissue/Disease Source	Deep perirenal adipose tissue
Product Description	hTERT immortalized brown preadipocytes were isolated from deep perirenal adipose tissue from a donor with von Hippel-Lindau syndrome.
Application	This hTERT-immortalized primary cell has applications as an in vitro cell model for toxicity studies and the study of obesity and related diseases.

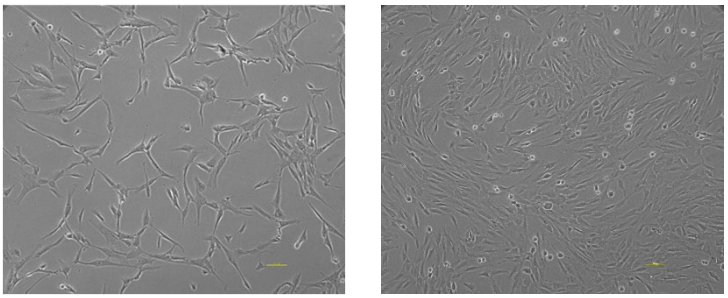

Figure 1: Cell morphology of Immortalized Brown Preadipocytes. Cells were maintained in ATCC recommended culture conditions. High and low confluence images of plated adherent brown preadipocytes were taken using Nikon microscope at 10x. Scale bar represents 100 microns.

Preadipocyte Marker Analysis

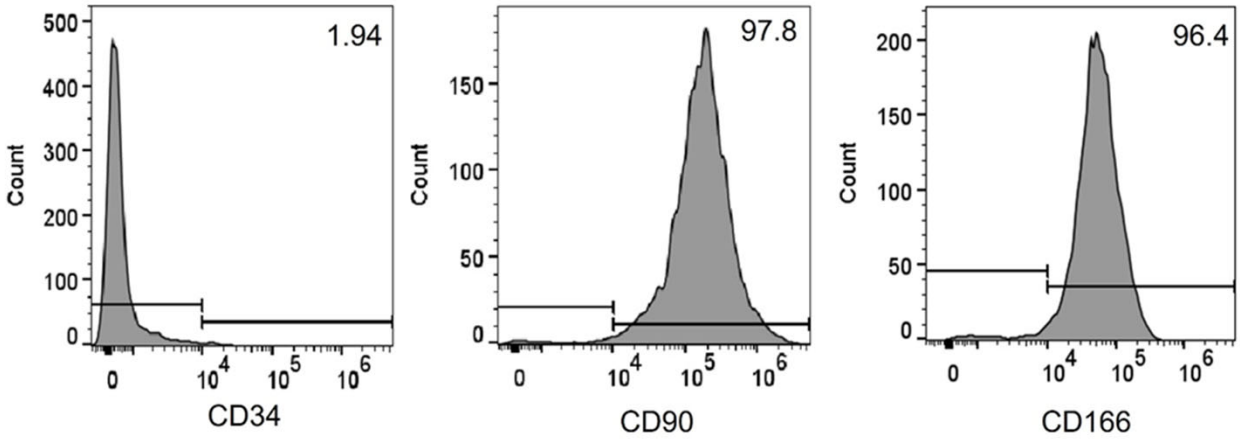

Figure 2: Marker expression by flow cytometry. Cells were stained for CD90 and CD166 which are common markers for cells of mesenchymal stem cell origin, and CD34, a marker for hematopoietic stem cells. As expected, brown preadipocytes were positive for CD90, CD166, but negative for CD34.

Differentiation of Brown Preadipocytes into Mature Adipocytes

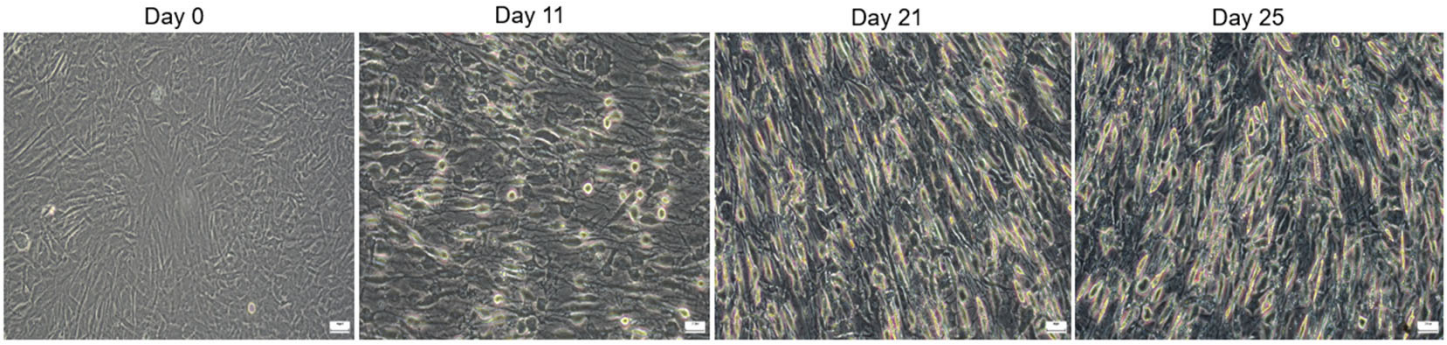

Figure 3: Differentiation of hTERT Brown Preadipocytes from day 0 through day 25. Images show the morphology of differentiating cells at different timepoints.

Protocol:

- Culture cells to reach around 70% confluent, seed cells into 6 well plates at a seeding density of 100k cells/cm^2.
- Observe cells under a phase contrast microscope daily until cells are 100% confluent. Wait 48 additional hours then begin differentiation/induction.
- Replace the cell culture media with Differentiation Media. Incubate the cells for 6 days.
- Use Induction Media 1 to replace the Differentiation Media. Let the cells grow for 7 days.
- Switch to Induction Media 2. Grow the for additional 7-12 days until Oil droplets can be seen clearly in the mature adipocytes.

Media Formulations:

Differentiation Media:

- ATCC DMEM F-12
- 2% ATCC FBS
- 1% Pen/Strep
- 0.5 uM Human Insulin
- 2 nM T3
- 3.3 nM BMP7

Induction Media 1:

- Advanced DMEM
- 2% Sigma FBS
- 1% Pen/Strep
- 0.5 uM Human Insulin
- 0.1uM Dexamethasone
- 0.5 mM IBMX
- 33 uM Biotin
- 2 nM T3
- 30 uM Indomethacin
- 17 uM Pantothenate
- 2 uM Rosiglitazone

Induction Media 2:

- Advanced DMEM
- 2% Sigma FBS
- 1% Pen/Strep
- 0.5 uM Human Insulin
- 0.1uM Dexamethasone
- 0.5 mM IBMX
- 33 uM Biotin
- 2 nM T3
- 30 uM Indomethacin
- 17 uM Pantothenate