

## Product Information and Certificate of Analysis

### Human Embryonic Stem (hES) Cell Line MEL-2

#### Product Description

Originating from Australia, the MEL-2 cell line was derived by Stem Cell Sciences Ltd. in a collaboration with Melbourne IVF in an NHMRC-licensed project (Licence No. 309709) from 'excess' human embryos donated by couples having IVF treatment. This line is registered on the USA's National Institute of Health's Human Embryonic Stem Cell Registry.

#### Properties

<b>Cell line</b>	MEL-2
<b>Cell type</b>	Human embryonic stem cells
<b>Gender</b>	Female
<b>Bank designation</b>	Working Cell Bank (WCB) #1
<b>Storage conditions</b>	≤ -135°C
<b>Date of cryopreservation</b>	10-Sep-2021
<b>Passage number</b>	P32
<b>Cell number</b>	5 × 10 <sup>6</sup> cells per vial
<b>Cryopreservation medium</b>	CryoStor® CS10
<b>Culture medium</b>	mTeSR™ Plus
<b>Matrix substrate</b>	Matrigel® - hESC-Qualified Matrix
<b>Recommended passage method and split ratio</b>	ReLeSR™, cells can generally be split 1:4-1:6 every 5 days

#### Recommended Materials for Use

Reagent	Supplier	Cat. Number
ReLeSR™	STEMCELL Technologies	100-0484
mTeSR™ Plus Kit	STEMCELL Technologies	05825
RevitaCell Supplement (100X)	Thermo Fisher Scientific	A2644501
Corning® Matrigel® hESC-Qualified Matrix	Corning®	354277
Accutase™	Thermo Fisher Scientific	A1110501

## CERTIFICATE OF ANALYSIS

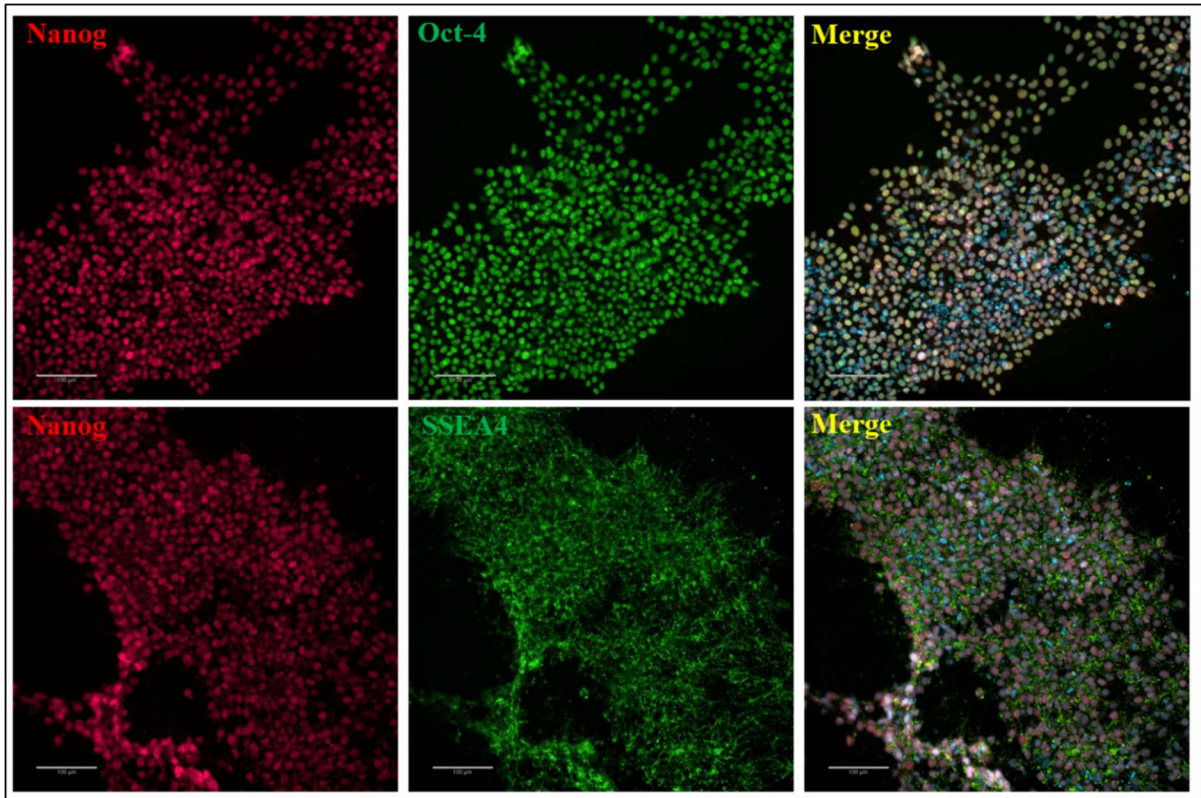
The following testing specifications have been met for this product:

Test Description	Test Method	Test Results
<b>Post-thaw cell viability and recovery</b>	Cell count	Viable cell count at revival > 80%. Cells reached confluence within 5 days.
<b>Mycoplasma</b>	Bioluminescence (Lonza MycoAlert detection kit)	Negative
<b>Expression of pluripotent stem cell markers</b>	Immunofluorescence	Positive for Oct-4, Nanog and SSEA4
	Flow cytometry	> 90% positive (SSEA4 and Tra-1-60)
	Gene expression by qPCR	Similar expression levels of Nanog, Oct-4, DNMT3B and Rex1 compared to pluripotent control
<b>Cytogenetics</b>	G-banding (500 band level)	Normal - 46, XX
<b>Genetic analysis</b>	Copy number variation test qPCR	Normal
<b>Differentiation potential</b>	Immunofluorescence	Positive for lineage specific markers: Ectoderm (Nestin, Pax6) Mesoderm (NCAM1, Brachyury) Endoderm (FOXA2, Sox17)
	Gene expression by qPCR	Increased expression of lineage-specific markers: Ectoderm (PAX6, SOX1, NR2F2) Mesoderm (HAND1, Brachyury) Endoderm (FOXA2, SOX17, Eomes, Gata6)

## Expression of Pluripotent Stem Cell Markers

### 1. Immunofluorescence

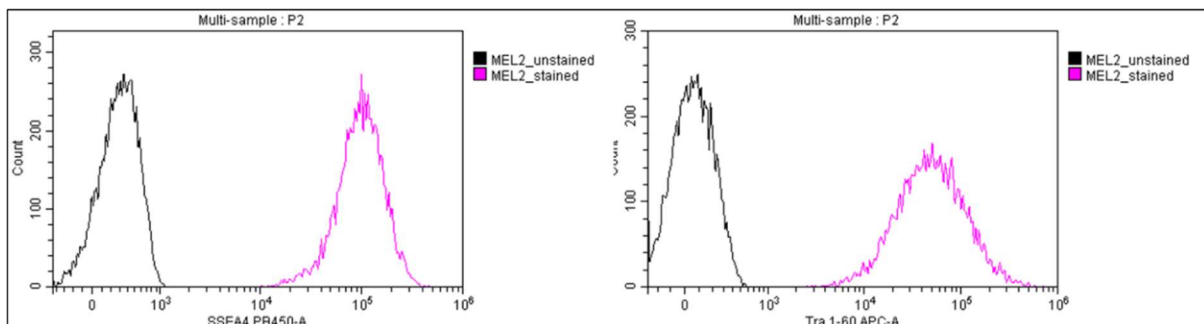
To assess pluripotency, MEL-2 cells were stained for nuclear markers Nanog and Oct-4, and surface marker SSEA4.



**Figure 1.** Pluripotency marker expression in MEL-2 WCB #1 cells demonstrating the presence of Nanog, Oct-4 and SSEA4. HB: Hoechst blue; Scale bar 100  $\mu$ m.

### 2. Flow Cytometry

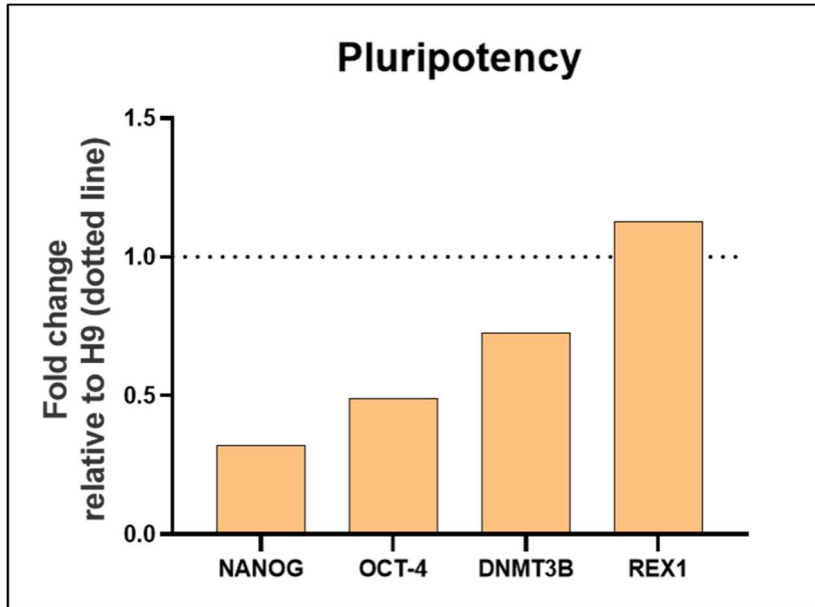
In addition to immunofluorescence, flow cytometry analysis confirms the presence of surface markers characteristic of pluripotent cells - SSEA4 and Tra-1-60.



**Figure 2.** Pluripotency marker expression as assessed by flow cytometry confirming the presence of SSEA4 and Tra-1-60. Black histograms are unstained samples overlaid with stained samples for MEL-2 WCB #1 cells.

### 3. Gene Expression Analysis

RNA of pluripotent cells is extracted and gene expression is measured by quantitative RT-PCR. GAPDH was used as the endogenous housekeeping gene. Relative gene expression is shown as fold difference in expression as compared to a pluripotent control (WA09, WiCell line H9). Pluripotency markers in MEL-2 WCB #1 are not more than 3-fold different to H9 control.

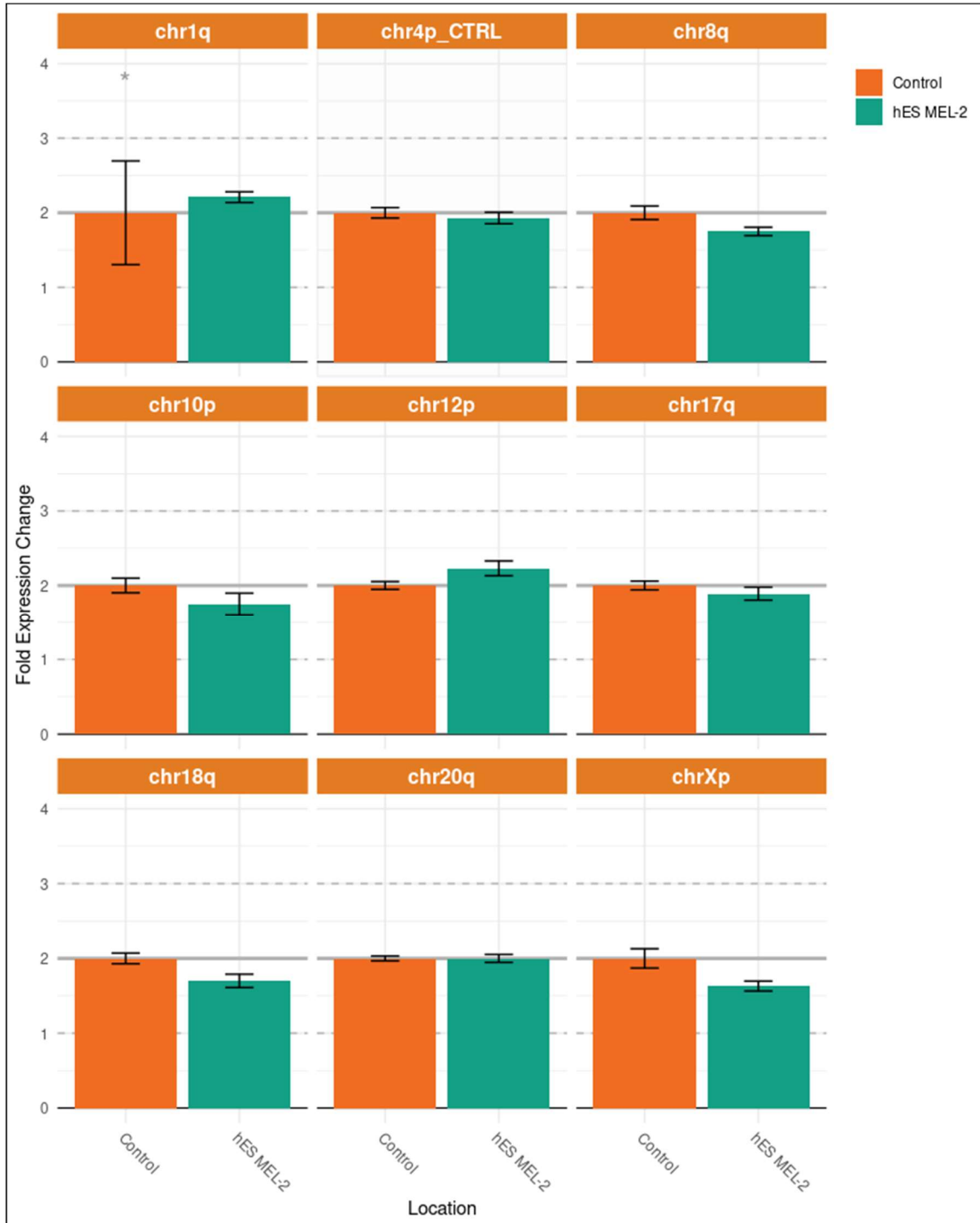


**Figure 3.** Gene expression analysis of pluripotency markers in MEL-2 cells demonstrating the expression of Nanog, Oct-4, DNMT3B and Rex1. The hES line H9 (WiCell) is used as positive pluripotent control (dotted line).

## Genetic Analysis

### 1. Copy Number Variation Test

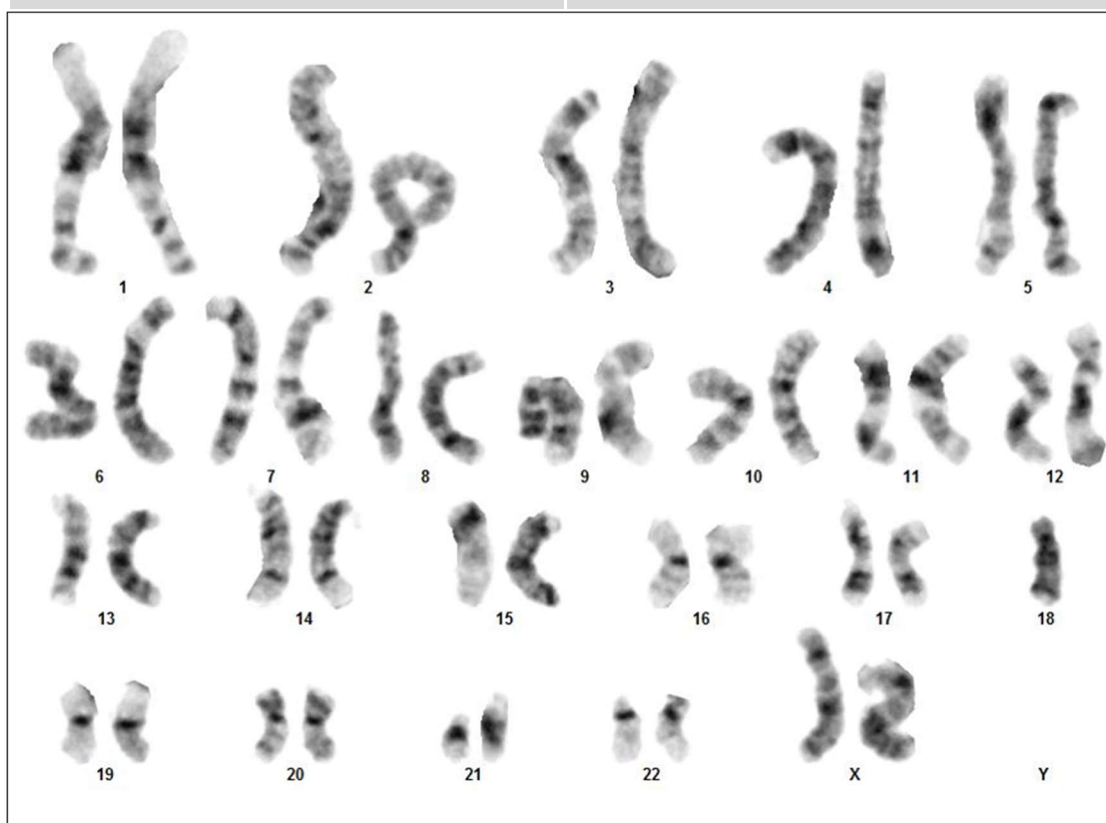
Genomic DNA from MEL-2 is screened for the 8 most common karyotypic abnormalities reported in human pluripotent stem cells using the hPSC Genetic Analysis Kit (STEMCELL Technologies).



**Figure 4.** Genetic abnormality assessment showing that all tested loci are normal in MEL-2 WCB #1 cells.

## 2. Cytogenetics

Test Method	G-Banding
G-band level	500
Passage at analysis	P32
Metaphase cells counted	4
Metaphase cells analysed	2
Short ISCN	46, XX

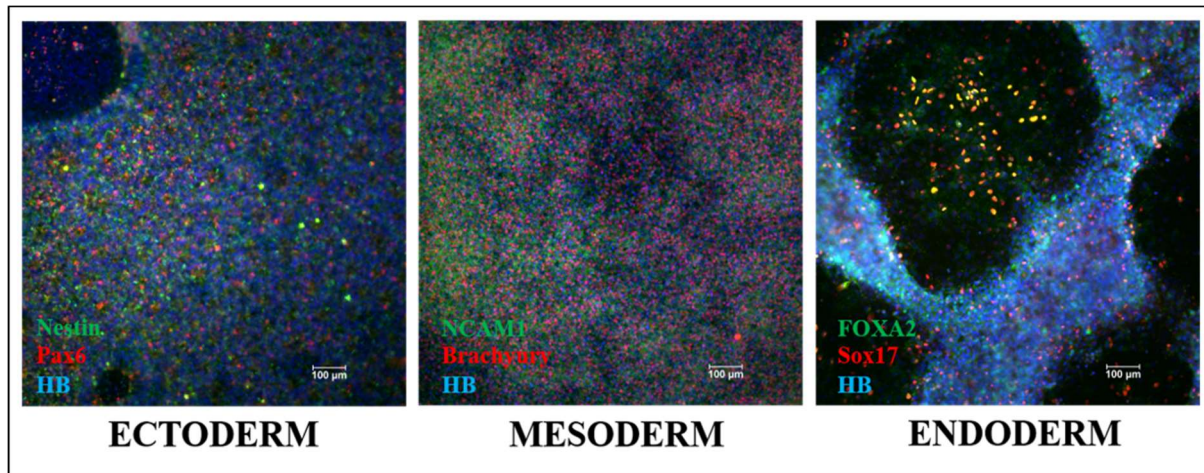


**Figure 5.** G-banding karyogram of MEL-2 WCB #1 cells showing a normal female karyotype – 46, XX (testing performed by Virtus Diagnostics).

## Differentiation Potential Analysis

### 1. Immunofluorescence

Cells are differentiated using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies) and stained for two markers from each germ layer.

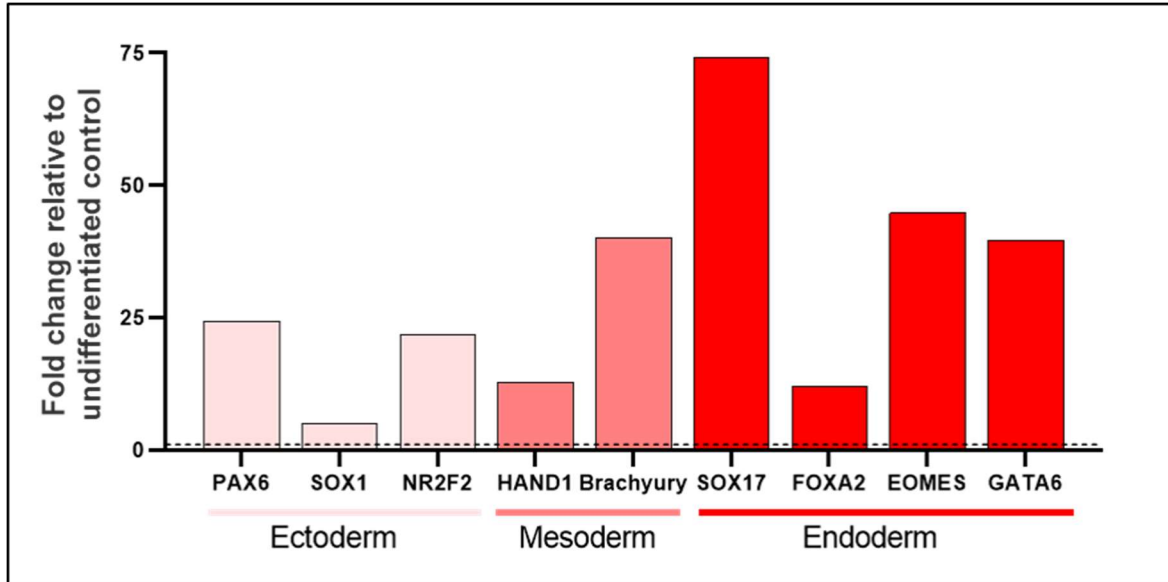


**Figure 6.** Trilineage differentiation of MEL-2 WCB #1 cells demonstrating the presence of markers from the three germ layers – Ectoderm (Nestin and Pax6), Mesoderm (NCAM1 and Brachyury) and Endoderm (FOXA2 and Sox17). HB: Hoechst blue; Scale bar 100 µm.

## 2. Gene Expression Analysis

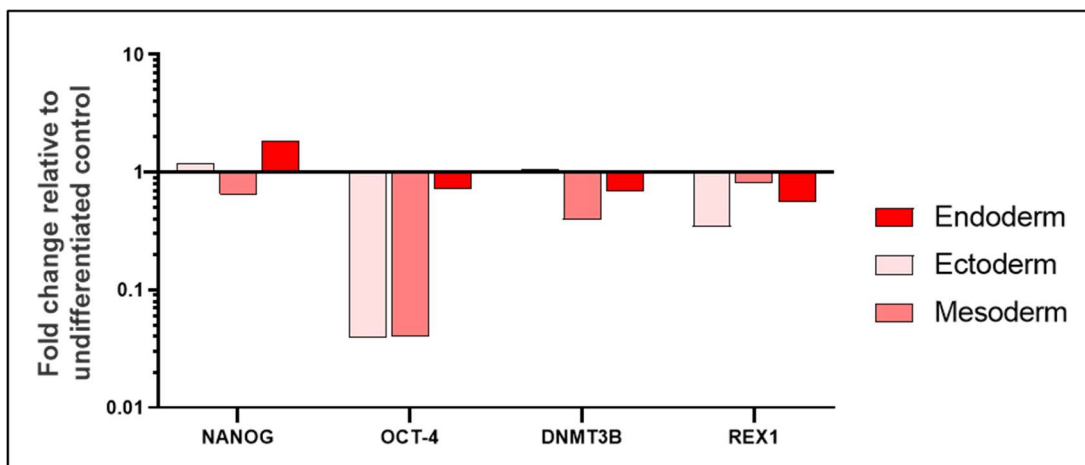
Cells are differentiated using the STEMdiff™ Trilineage Differentiation Kit (STEMCELL Technologies). RNA is extracted and gene expression is measured by quantitative RT-PCR. GAPDH is used as the endogenous housekeeping gene. Relative gene expression is shown as the fold difference in expression as compared to undifferentiated pluripotent MEL-2 cells. Calculations are performed using the  $2^{-\Delta\Delta CT}$  method. (Livak KJ, Schmittgen TD. Methods. 2001 Dec;25(4):402-8.PMID:11846609).

### Germ Layer Gene Expression During Lineage Differentiation



**Figure 7.** Gene expression analysis of differentiated MEL-2 WCB #1 cells demonstrating the expression of genes from the three germ layers - Ectoderm (PAX6, SOX1 and NR2F2), Mesoderm (HAND1 and Brachyury) and Endoderm (SOX17, FOXA2, EOMES and GATA6) relative to undifferentiated control (dotted line).

### Pluripotent Gene Expression During Lineage Differentiation



**Figure 8.** Gene expression analysis of differentiated MEL-2 WCB #1 cells demonstrating downregulation of pluripotency genes in the three germ layers.

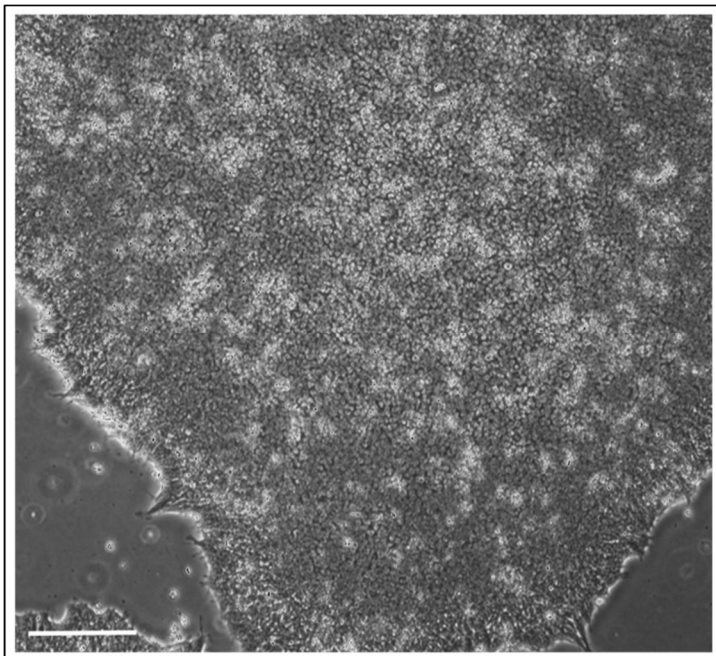


## Directions for Use

The following instructions are for preparing one cryovial of MEL-2 for plating in  $1 \times 75\text{cm}^2$  flask. Use aseptic technique throughout the protocol.

### Thawing and plating MEL-2

- 1) Coat culture-ware flask with Matrigel® (made to manufacturer's specifications) and incubate for at least 30 minutes at  $37^\circ\text{C}$  or overnight at  $4^\circ\text{C}$ . Prior to cell seeding, the flask should be pre-warmed to  $37^\circ\text{C}$  and the mTeSR™ Plus medium warmed to room temperature.
- 2) Gently warm the cryovial in a water bath until a small ice pellet remains and transfer into a biosafety cabinet.
- 3) Add 1 ml of mTeSR™ Plus media to cryovial and transfer content into a 15 ml centrifuge tube containing 2 ml of mTeSR™ Plus media.
- 4) Centrifuge at  $200 \times g$  for 3 minutes.
- 5) Add RevitaCell to mTeSR™ Plus medium according to manufacturer's specifications.
- 6) Aspirate the media avoiding disruption to the cell pellet and add 1 ml of mTeSR™ Plus with RevitaCell.
- 7) Remove the substrate coating prior to transferring the cells and add mTeSR™ Plus medium with RevitaCell to flask (11 ml/T75 flask).
- 8) Gently re-suspend the cell pellet and transfer to the Matrigel-coated flask containing mTeSR™ Plus and RevitaCell.
- 9) Transfer flask into an incubator at  $37^\circ\text{C}$  and do not disturb for 24 hours.
- 10) Perform a full media exchange 24 hours later (without RevitaCell) and then replace daily or every second day (depending on cell confluence).



**Figure 9.** MEL-2 colony at Day 4 post-recovery from cryostorage. Scale bar 200  $\mu\text{m}$ .

### Passaging:

- 1) When cells reach 80-90% confluence prepare the following reagents and volumes per T75 flask (adjust volumes as required for specific culture flask):
  - a) 5 ml ReLeSR™ (pre-warm to 37°C).
  - b) 10 ml of PBS (calcium and magnesium free (CMF), pre-warm to 37°C).
  - c) T75 flasks pre-coated with extracellular matrix (ECM) of choice according to manufacturer's instructions.
  - d) 15 ml mTeSR™ Plus (pre-warm to 37°C) containing 1x RevitaCell at recommended dilution (or 10 µM ROCK inhibitor).
  - e) 5 ml of DMEM (pre-warm to 37°C, specific formulation not strictly important).
- 2) Remove flask from incubator and transfer to biosafety cabinet.
- 3) Aspirate media and wash cells with 10 ml PBS CMF. Aspirate PBS.
- 4) Add 5 ml of ReLeSR™. Lay down flask and ensure entire surface is covered by ReLeSR™.
- 5) Incubate in biohazard hood for no more than 30 seconds.
- 6) Aspirate ReLeSR™ and immediately transfer flask to 37°C incubator.
- 7) Incubate for 2 min. Check cells under microscope for rounding of cells. Incubate for 1 minute longer if required.
- 8) In the biosafety cabinet, add 5 ml of DMEM down the side of the flask.
- 9) Tilt flask so surface is covered with DMEM and tap the side of the flask 2 or 3 times. If incubation time in ReLeSR™ is sufficient, cells will lift off with tapping.
- 10) Wash the surface with the 5 ml DMEM/cell suspension and transfer to a centrifuge tube.
- 11) Pellet the cells at 300 × g for 3 minutes.
- 12) Aspirate supernatant and add 1 ml of mTeSR™ Plus with RevitaCell. Mix well to resuspend pellet.
- 13) Aspirate ECM from T75 and immediately add 12 ml of mTeSR™ Plus with RevitaCell down the side of the flask to avoid removing ECM from surface.
- 14) Take 1:6 to 1:8 of the cell suspension and transfer to one T75 with media.
- 15) Transfer to CO<sub>2</sub> incubator. Gently agitate flask back and forth to ensure even spread of cells in the flask.
- 16) Change media 24 hrs later to remove RevitaCell. 12-20 ml per T75 flask is recommended based on confluence at the time.
- 17) Cells will reach confluence within 4-5 days.

### **Cryopreservation:**

- 1) Follow the passaging protocol up to step 10.
- 2) Take a small aliquot of the cell suspension (volume of your choice) and transfer to a second centrifuge tube to perform a cell count.
- 3) Centrifuge both tubes at  $300 \times g$  for 3 minutes.
- 4) Aspirate media and resuspend cell suspension for cryopreservation in 5 ml of mTeSR™ Plus.
- 5) Resuspend cell count aliquot in 1 ml of pre-warmed Accutase™ (include a PBS CMF wash before this if desired. Cells are currently in small clusters of 5 - 10 cells and don't require much effort for single cell dissociation).
- 6) Incubate at room temperature for 3 minutes with regular gentle agitation using a 200  $\mu$ l pipette.
- 7) Perform cell count using desired method.
- 8) Back calculate to determine cell number of original cell suspension.
- 9) Centrifuge cells at  $300 \times g$  for 3 minutes.
- 10) Aspirate mTeSR™ Plus.
- 11) Gently flick tube to dislodge cell pellet.
- 12) Add cold, CryoStor® CS10 to a final cell density of  $1 - 5 \times 10^6$  cells/ml. We recommend not cryopreserving densities lower than  $1 \times 10^6$  cells/ml. Higher densities improve cell recovery at thaw.
- 13) Aliquot  $\geq 500 \mu$ l of cells in CryoStor® CS10 per cryovial.
- 14) Transfer to isopropanol tanks and then to  $-80^\circ\text{C}$  for 2 hours before transfer to cryostorage.
- 15) Depending on confluence, each T75 flask contains  $18 - 25 \times 10^6$  cells.