Data Sheet

O9-1 Mouse Cranial Neural Crest Cell Line

Cell Line

SCC049

Pack Size: ≥1 x 10^6 cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Cranial neural crest cells give rise to ectomesenchymal derivatives such as cranial bones, cartilage, smooth muscle, dentin, as well as melanocytes, corneal endothelial cells, and neurons and glial cells of the peripheral nervous system. Previous studies have suggested that although multipotent stem-like cells may exist during the course of cranial neural crest development, they are transient, undergoing lineage restriction early in embryonic development.

Whole-genome expression profiling of O9-1 cells revealed that this line stably expresses stem cell markers (CD44, Sca-1, and Bmi1) and neural crest markers (AP-2α, Twist1, Sox9, Myc, Ets1, Dlx1, Dlx2, Crabp1, Epha2, and Itgb1). O9-1 cells are capable of contributing to cranial mesenchymal (osteoblast and smooth muscle) neural crest fates when injected into E13.5 mouse cranial tissue explants and chicken embryos. These results suggest that O9-1 cells represent multipotent mesenchymal cranial neural crest cells. The O9-1 cell line should serve as a useful tool for investigating the molecular properties of differentiating cranial neural crest cells. The O9-1 cell line can be propagated and passaged for at least 10 passages, and can differentiate into osteoblasts, chondrocytes, smooth muscle cells, and glial cells.[1]

Source

Derived from mass cultures of Wnt1-Cre; R26R-GFP reporter-expressing cranial neuronal crest cells from E8.5 mouse embryos.

Quality Control Testing

- Each vial contains ≥1 x 10^6 viable cells at passage 20–22.
- Cells tested negative for infectious disease by a murine PCR panel (Mouse Essential CLEAR Panel by Charles River Animal Diagnostic Services).
- Cells tested negative for *mycoplasma* contamination.

Storage and Handling

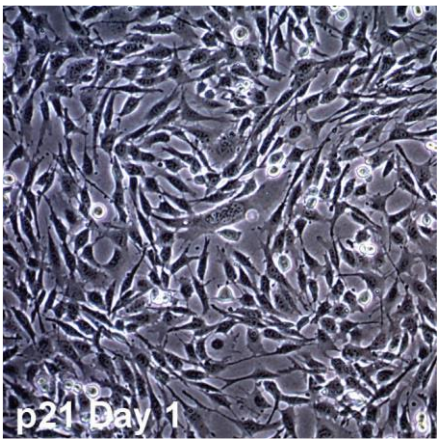
O9-1 cells should be stored in liquid nitrogen. The cells can be passage for at least 10 passages without significantly affecting the cell marker expression and functionality.

Materials Required (Not provided)

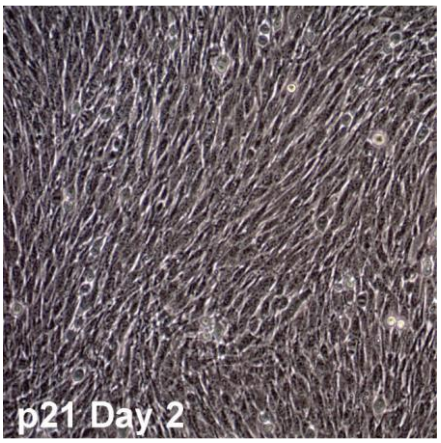
- Complete ES Cell Medium with 15% FBS and LIF (ES-101-B)
- Recombinant Human bFGF (GF003)
- Matrigel® Membrane Matrix (Corning® Matrigel® Basement Membrane Matrix LDEV-Free, CLS356234)
- Accumax™ Solution (SCR006)
- Trypsin-EDTA (SM-2003-C)
- Dulbecco's Phosphate-Buffered Saline, 1X ES Cell Qualified (D8537)
- Tissue culture-wares and supplies
- Cell counter/hemocytometer
- Microscope

Representative Data

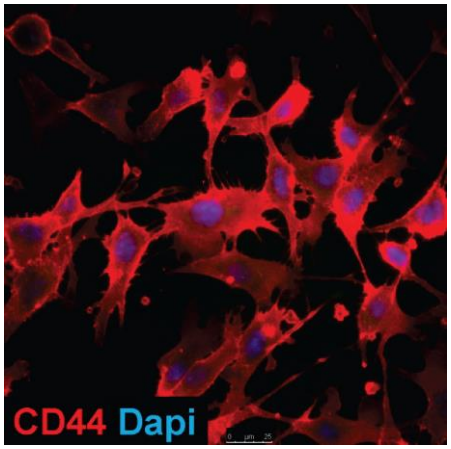
O9-1 Cells After Thaw



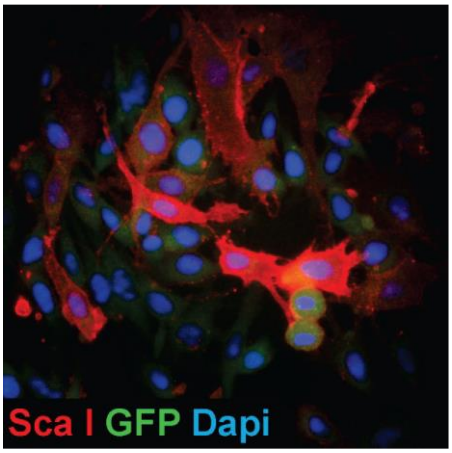
O9-1 Cells 24 Hours After Thaw



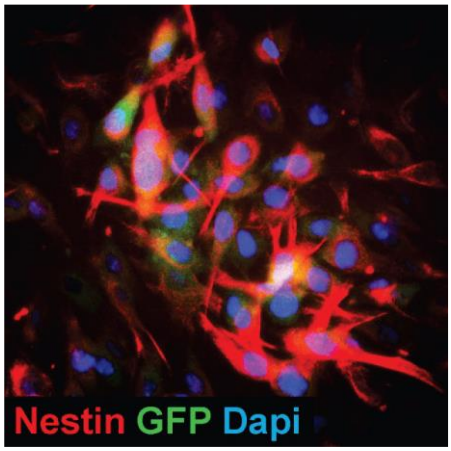
O9-1 Cells Express Stem Cell Markers



O9-1 Cells stained with anti-CD44 (MABT78, 1:200)

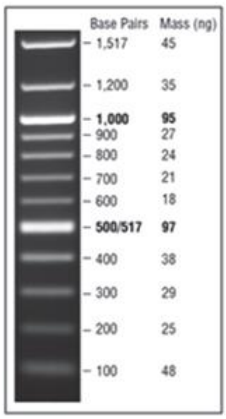


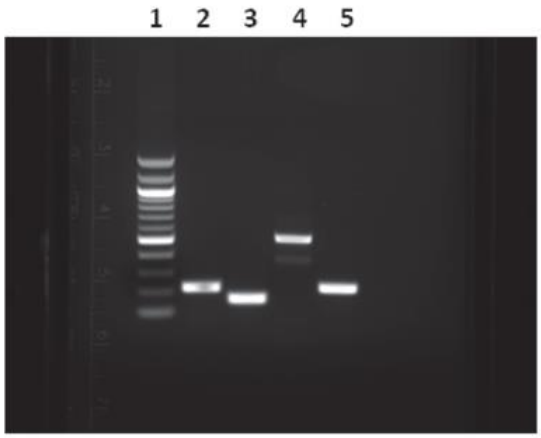
O9-1 Cells stained with anti-Sca-1 (MABD26, 5 µg/mL)



O9-1 Cells stained with anti-Nestin (MAB353, 5 µg/mL)

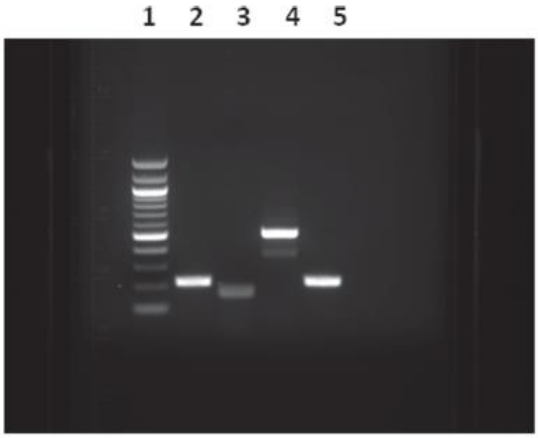
O9-1 Cells Express Neural Crest Markers





O9-1 Cells; Passage 31

1. 100 bp DNA Ladder (NEB, QuickLoad)
2. P31, Twist1, 37 cycles @ 62 °C, 25 bp
3. P31, Snail1, 37 cycles @ 56 °C, 167 bp
4. P31, AP-2α, 37 cycles @ 62 °C, 518 bp
5. P31, β-Actin, 30 cycles @ 56 °C, 222 bp



O9-1 Cells; Passage 21

1. 100 bp DNA Ladder (NEB, QuickLoad)
2. P21, Twist1, 37 cycles @ 62 °C, 225 bp
3. P21, Snail 1, 30 cycles @ 56 °C, 167 bp
4. P21, AP-2α, 37 cycles @ 62 °C, 518 bp
5. P21, β-Actin. 30 cycles @ 56 °C, 222 bp

Protocols

O9-1 Cell Medium Preparation

Supplement medium with 15% FBS, mLIF (ES-101-B), and 25 ng/mL FGF-2 (GF003). Only FGF-2 is needed if using Complete ES Cell Medium (ES-101-B). Sterile filter with 0.22 µm filter.

ECM Coating of Flasks

1. Thaw Matrigel® Membrane Matrix (CLS356234) on ice at 4 °C. Do not thaw Matrigel® Membrane Matrix at room temperature as this will lead to unwanted gelling.
2. Dilute Matrigel® solution 1:50 in chilled 1X PBS (BSS-1005-B) and maintain on ice.
3. Coat flasks with 1:50 diluted Matrigel® solution. Use 5–10 mL for each T75 flask and 15–25 mL for each T225 flask. Leave at room temperature for 1 hour before use.

   NOTE: Flasks may be coated 5–6 days in advance and stored at 2–8 °C.
4. Aspirate the coating solution just before plating the cells.

Thawing of Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells are thawed in O9-1 Cell Medium (See O9-1 Cell Medium Preparation).
2. Remove the vial of O9-1 Mouse Cranial Neural Crest 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 O9-1 Cell Medium (See O9-1 Cell Medium Preparation), pre-warmed to 37 °C, to the 15 mL conical tube.

   IMPORTANT: Do not add the entire volume of media 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 to not 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 a total volume of 10–12 mL O9-1 Cell Medium (See O9-1 Cell Medium Preparation) pre-warmed to 37 °C.
10. Plate the cell mixture onto a precoated T75 tissue culture flask (See ECM Coating of Flasks).
11. Maintain the cells at 37 °C in a 5% CO_2 humidified incubator.
12. The next day, replace the medium with fresh O9-1 Cell Medium (pre-warmed to 37 °C). Replace with fresh medium every two to three days thereafter.
13. When the cells are approximately 80% confluent (3-4 days after plating cells), they can be dissociated with Accumax™ solution (SCR006) or trypsin-EDTA (SM-2003-C) and passaged further or frozen for later use.

Subculturing of Cells

1. Carefully remove the medium from the T75 tissue culture flask containing the confluent layer of O9-1 Mouse Cranial Neural Crest Cells.
2. Apply 3–5 mL of Accumax™ or trypsin-EDTA solution and incubate in a 37 °C incubator for 3–5 minutes.
3. Inspect the plate and ensure the complete detachment of cells by gently tapping the side of the plate with the palm of your hand.
4. Add 8 mL of O9-1 Cell Medium (pre-warmed to 37 °C) to the plate.
5. Gently rotate the plate to mix the cell suspension. Transfer the dissociated cells to a 15 mL conical tube.
6. Centrifuge the tube at 300 x *g* for 3-5 minutes to pellet the cells.
7. Discard the supernatant.
8. Apply 2 mL of O9-1 Cell Medium (pre-warmed to 37 °C) to the conical tube and resuspend the cells thoroughly.
   IMPORTANT: Do not vortex the cells.
9. Count the number of cells using a hemocytometer.
10. Plate the cells to the desired density (typical split ratio is 1:3 to 1:6).

Cryopreservation of Cells

O9-1 Mouse Cranial Neural Crest cells can be frozen in O9-1 Cell Medium (See O9-1 Cell Medium Preparation) with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Ishii, M., Arias, A. C., Liu, L., Chen, Y. B., Bronner, M. E., & Maxson, R. E. (2012). A stable cranial neural crest cell line from mouse. Stem cells and development, 21(17), 3069-3080.
2. Sun, J., Ishii, M., Ting, M.C. and Maxson, R., 2013. Foxc1 controls the growth of the murine frontal bone rudiment by direct regulation of a Bmp response threshold of Msx2. Development, 140(5), pp.1034-1044.

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