MilliTrace™
Nanog GFP Reporter
Mouse Embryonic Stem Cell Kit

Cat. No. SCR089

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Not for use in diagnostic procedures

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Introduction

MilliTrace™ Nanog GFP Reporter Mouse Embryonic Stem Cell Kit (Catalog No. SCR089) provides ready-to-use mouse embryonic stem cells that express the humanized mulleri green fluorescent protein (hmGFP) reporter gene under the control of the mouse Nanog promoter along with expansion medium to help maintain expression of the transgene.

Nanog is an important marker of undifferentiated embryonic stem cells and, along with Oct-4 and Sox-2, is an important regulator of stem cell pluripotency. Studies have shown that down-regulation of the Nanog promoter coincides with the loss of pluripotency and progression towards a differentiated state. Nanog GFP Reporter Mouse Embryonic Stem Cell Kit provides a quick non-invasive method by which to monitor the expression of Nanog in pluripotent embryonic stem cells. The cells can be used to facilitate studies elucidating the role of Nanog and other factors in the maintenance and self-renewal of embryonic stem cells. This kit can also be used to identify factors involved in embryonic stem cell differentiation.

MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells were generated by transfection of C57/BL6 mouse embryonic stem cells with a proprietary bicistronic plasmid construct containing hmGFP under the control of the mouse Nanog promoter. FACS analyses of stable transfectants indicate that greater than 90% of the cells express GFP at high levels even after 10 passages when cultured at normal proliferation conditions. MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells display the immunochemical staining properties of pluripotent stem cells; they are positive for alkaline phosphatase and Nanog-hmGFP expression is co-localized with Oct-4, Sox-2, and SSEA-1 expression. Upon differentiation, hmGFP is down-regulated and cells have the capacity to differentiate into multiple lineages, including cardiomyocytes and neurons. Under neuronal differentiation conditions, cells can be differentiated to βIII-tubulin positive neurons (GFP^+ $\beta III\text{-}tubulin^+$) (Use Mouse Embryonic Stem Cell Neurogenesis Kit, Catalog No. SCR101). Cells have been confirmed to be mycoplasma-free and demonstrate an apparently normal karyotype (40, XY) as assessed by standard G-banding analysis performed on twenty metaphase cells.

We recommend that the MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cell Kit (Catalog No. SCR089) be used in conjunction with the Embryonic Stem Cell Marker Characterization Kit (Catalog No. SCR001) and differentiation assays that demonstrate pluripotentiality of the starting cell population, Mouse Embryonic Stem Cell Neurogenesis Kit (Catalog No. SCR101) and Mouse Embryonic Stem Cell Adipogenesis Kit (Catalog No. SCR100).

We do not recommend use of the MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells to generate GFP-labeled mouse through germ line transmission.

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Kit Components

1. 1 x 10^6 viable MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells: (Catalog No. SCC089) derived from C57/BL6 embryonic stem cell line (Catalog No. CMTI-2), cryopreserved. Store in liquid nitrogen.

2. MilliTrace Mouse Embryonic Stem Cell Expansion Medium: (Catalog No. SCM042) contains 500 mL Complete ES Cell Media with 15% FBS and LIF (Catalog No. ES-101-B) and 100 μL of a 5 mg/mL Puromycin Solution (Part No. CS201361). Store at -20°C.

Characterization of Cells

MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells have been validated for expression of hmGFP in the pluripotent state, for high level expression of alkaline phosphatase, Oct-4, Sox-2 and SSEA-1, and for their self-renewal and multi-lineage differentiation capacities (please refer to product manual figures for representative data). Approximately 80% cells display the normal karyotype as assessed by G-banding of 20 metaphase cells. Cells tested negative for mycoplasma.

Materials Required But Not Provided

1. EmbryoMax ES Cell Qualified 0.1% Gelatin Solution, 500 mL (Catalog No. ES-006-B)

2. Accutase™ Cell Dissociation Solution (Catalog No. SCR005)

3. Tissue culture-ware

4. Phosphate-Buffered Saline (1X PBS) (Catalog No. BSS-1005-B)

5. EmbryoMax ES Cell Qualified Ultra Pure Water, sterile H_20, 500 mL (Catalog No. TMS-006-B)

6. Fixative (e.g. 4% Paraformaldehyde in 1X PBS)

7. Blocking Solution (5% normal donkey serum, 0.3% Triton X-100 in 1X PBS)

8. Non-Permeable Blocking Solution (5% normal donkey serum in 1X PBS)

9. Fluorescent-labeled secondary antibodies. Donkey anti-mouse IgG, Cy3 conjugated (Catalog No. AP192C), donkey anti-mouse IgM, Cy3 conjugated (Jackson Laboratories Catalog No. 715-165-140) and donkey anti-rabbit IgG, Cy3 conjugated (Catalog No. AP182C) are recommended

10. Isotype controls (e.g. mouse IgG (Catalog No. PP54), mouse IgM (Catalog No. PP50), and rabbit IgG (Catalog No. PP64))

11. 4'-6-Diamidino-2-phenylindole (DAPI) / PBS solution

12. Tryphan Blue

13. Nunc Lab-Tek II 8 well chamber slides (Fisher Catalog No. 12-565-8)

14. Anti-fading mounting solution (DABCO/PVA)

15. Hemacytometer

16. Microscope with appropriate fluorescent filters

Storage

MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells (Part No. SCC089) should be stored in liquid nitrogen. We recommend that the cells be used within ten passages.

EmbryoMax Complete ES Cell Media w/ 15% FBS and LIF (Part No. ES-101-B) should be stored at -20°C until expiration date on the label. Upon thawing the basal medium should be stored at 2-8°C and given a one month expiration dating.

5 mg/mL Puromycin Solution, 100 µL (Part No. CS201361) should be stored in working aliquots at -20°C for up to 1 year.

Preparation of Coated Plates

We recommend coating tissue culture plastic- or glassware that are used to culture mouse embryonic stem cells with 0.1% gelatin. The following procedure is recommended:

1 Add enough of the 0.1% gelatin solution (Cat. No. ES-006-B) to cover the whole surface of the tissue cultureware. Use 5 mL volume for 6-cm plates and 10 mL volume for 10-cm plates and T75 flasks. Incubate for at least 30 minutes at room temperature.

2. Just before use, aspirate the gelatin solution from the coated plate.

Thawing of Cells

1. Do not thaw the cells until the recommended medium and appropriately coated 0.1% gelatin plasticware and/or glassware are on hand.

2. Remove the vial of MilliTrace Nanog GFP Reporter Mouse Embryonic Stem 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 to not introduce any bubbles during the transfer process.

5. Using a 10 mL pipette, slowly add dropwise 9 mL Complete ES Media with 15% FBS and LIF (Catalog No. ES-101-B) (pre-warmed to 37°C) to the 15 mL conical tube. **IMPORTANT: Do not add the whole 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 slow pipeting up and down twice. Be careful to not introduce any bubbles. IMPORTANT: Do not vortex the cells.

7. Centrifuge the tube at 300 xg 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 mL Complete ES Cell Media with 15% FBS and LIF (Catalog No. ES-101-B) (pre-warmed to 37°C) containing 0.5 μg/mL puromycin.

   Note: *Puromycin should always be added fresh to the Complete ES Cell Media with 15% FBS and LIF. To obtain final concentrations of 0.5 μg/mL puromycin, add 1 μL of puromycin stock to 10 mL of Complete ES Cell Media with 15% FBS and LIF.*

10. Plate the cell mixture onto a gelatin-coated 10-cm tissue culture plate.

11. Incubate the cells at 37°C in a 5% CO_2 humidified incubator.

12. The next day, exchange the medium with fresh Complete ES cell Media with 15% FBS and LIF Medium (pre-warmed to 37°C) containing 0.5 μg/mL puromycin. Exchange with fresh medium containing puromycin every other day thereafter.

13. When the cells are approximately 80% confluent, they can be dissociated with Accutase Cell Dissociation Solution (Catalog No. SCR005) and passaged or alternatively frozen for later use.

Subculturing

1. Carefully remove the medium from the gelatin-coated 10-cm tissue culture plate containing the confluent layer of MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells.

2. Apply 3-5 mL of Accutase and incubate in a 37°C incubator for 3 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. Apply 5 mL Complete ES Cell Media with 15% FBS and LIF (pre-warmed to 37°C) to the plate.

5. Transfer the dissociated cells to a 15 mL conical tube.

6. Centrifuge the tube at 300 xg for 2- 3 minutes to pellet the cells.

7. Discard the supernatant.

8. Apply 2 mL Complete ES Cell Media with 15% FBS and LIF containing 0.5 μg/mL puromycin to the conical tube and resuspend the cells thoroughly.

9. Count the number of cells using a hemacytometer.

10. Plate the cells to the desired density into the appropriate gelatin-coated flasks, plates or wells in Complete ES Cell Media with 15% FBS and LIF containing 0.5 μg/mL puromycin.

Results

Characterization of MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells (Catalog No. SCC089)

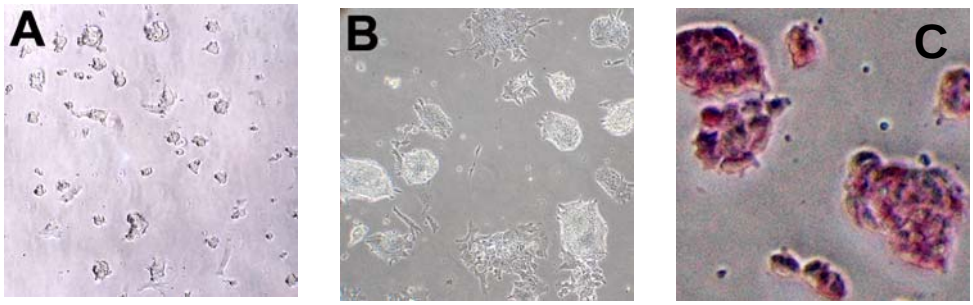


Figure 1. Phase contrast images of MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells (Catalog No. SCC089) one (**A**) and two (**B**) days after thawing. Cells are strongly positive for alkaline phosphatase (**C**). Cells were cultured on 0.1% gelatin coated 10-cm tissue culture plates in the absence of mouse feeder cells.

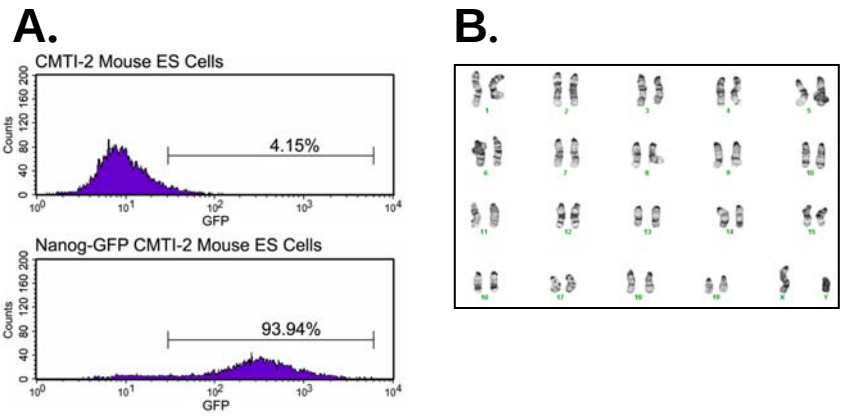


Figure 2. FACS analysis of MilliTrace Nanog GFP Reporter Mouse Embryonic Stem Cells using a Guava flow cytometer indicates that > 90% ESCs express GFP (**A**). Approximately 80% of cells have a normal male karyotype (40, XY) as assessed by G-banding of twenty metaphase cells (**B**).

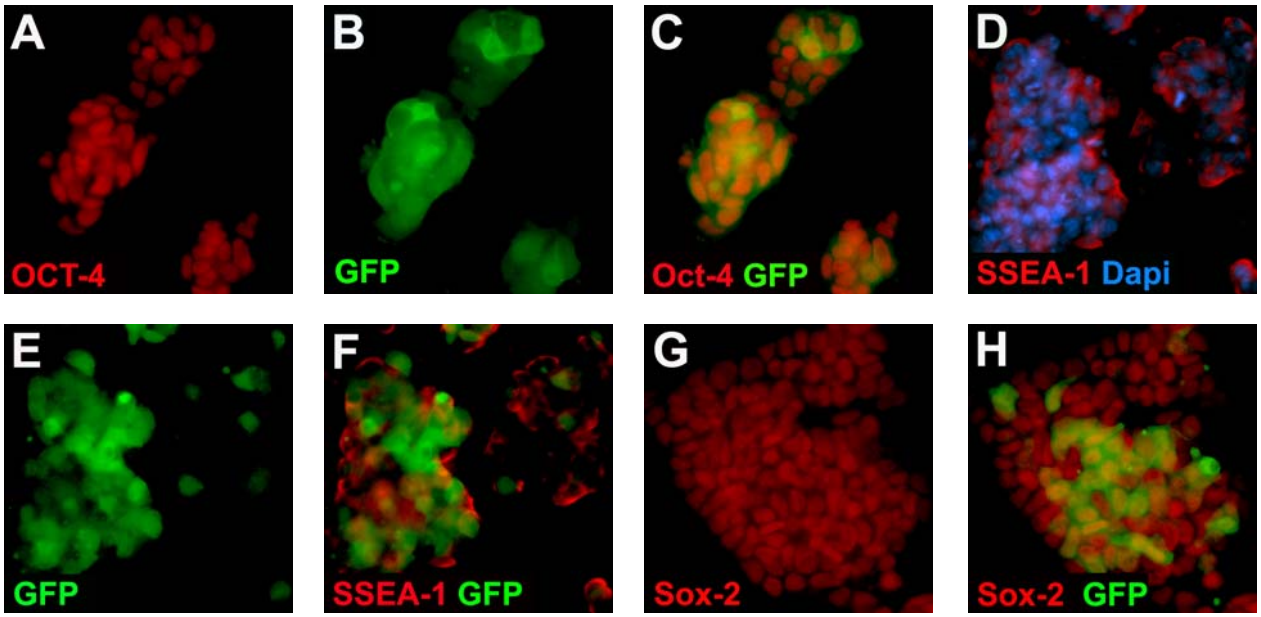
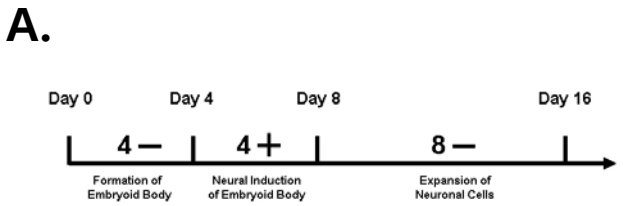


Figure 3. MilliTrace Nanog Reporter Mouse Embryonic Stem Cells (Catalog No. SCC089) express GFP (**B, E)** and the pluripotency markers, OCT-4 (**A**, red), SSEA-1 (**D**, red), and Sox-2 (**G**, red). Merged images of GFP expression driven by the Nanog transcription factor with pluripotency markers (**C, F, H),**



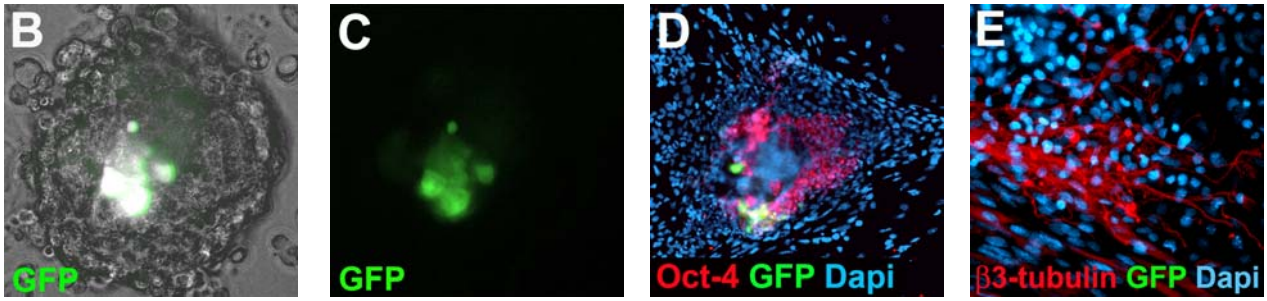


Figure 4. Downregulation of Nanog-hmGFP expression after 8 days (**B**, **C**, **D**) and 16 days (**E**) of neuronal differentiation. Expression of the pluripotency marker OCT-4 is also downregulated (**D**, red) after 8 days of differentiation. After 16 days of differentiation, Nanog-hmGFP expression is completely absent and there is a concomitant increase in neuronal expression (βIII-tubulin, red, **E**). Mouse ES cells were cultured in EB Formation Medium (Catalog No. SCM018) in a non-adhesive 10-cm Petri dish for 4 days in the absence of LIF (4- Condition) to form embryoid bodies (EBs) (**A**). Retinoic acid was added to the culture medium for 4 days (4+ Condition) to induce neuronal differentiation. Neural induced EBs were then transferred to poly-L-ornithine and laminin coated 8-well chamber slides and cultured in EB Formation Medium for an additional 8 days (8- Condition)

*For color images, please go to www.millipore.com

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Related Products

The following products are available from Millipore as separate items:

1. Mouse Embryonic Stem Cell Neurogenesis Kit: (Catalog No. SCR101)
2. Mouse Embryonic Stem Cell Adipogenesis Kit: (Catalog No. SCR100)
3. Embryoid Body (EB) Formation Medium: (Catalog No. SCM018)
4. Laminin, mouse: (Catalog No. CC095)
5. EmbryoMax Complete ES Cell Media w/ 15%FBS and LIF: (Catalog No. ES-101-B)
6. Embryonic Stem Cell Marker Characterization Kit: (Catalog No. SCR001)
7. Alkaline Phosphatase Detection Kit: (Catalog No. SCR004)
8. Quantitative Alkaline Phosphatase ES Cell Characterization Kit: (Catalog No. SCR066)
9. Mouse anti-SSEA-1, 100 μg: (Catalog No. MAB4301)
10. Rabbit anti-Sox-2, 100 μg: (Catalog No. AB5603)
11. Mouse anti-βIII tubulin, 100 μL: (Catalog No. MAB1637)
12. Mouse IgM, purified 1 mg: (Catalog No. PP50)
13. Mouse IgG, purified 10 mg: (Catalog No. PP54)
14. Rabbit IgG, purified 25 mg: (Catalog No. PP64)

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