# MilliTrace™
# Constitutive GFP Reporter
# Mouse Embryonic Stem Cell Kit

**Cat. No. SCR082**

**FOR RESEARCH USE ONLY**
**Not for use in diagnostic procedures**

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## Introduction

MilliTrace™ Constitutive GFP Reporter Mouse Embryonic Stem Cell Kit (Catalog No. SCR082) provides ready-to-use mouse embryonic stem cells that are constitutively labeled with the humanized mulleri green fluorescent protein (hmGFP) along with expansion medium to help maintain expression of the transgene.

MilliTrace Constitutive 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 a constitutive chicken actin promoter. FACS analyses of stable transfectants indicate that greater than 95% of the cells express GFP at high levels even after 10 passages. MilliTrace Constitutive GFP Reporter Mouse Embryonic Stem Cells display the immunochemical staining properties of pluripotent stem cells and are thus positive for alkaline phosphatase, Oct-4, Sox-2, and SSEA-1. Under neuronal differentiation conditions, cells can be differentiated to βIII-tubulin positive neurons ($GFP^+$βIII-$tubulin^+$) (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 Constitutive GFP Reporter Mouse Embryonic Stem Cell Kit (Catalog No. SCR082) be used in conjunction with the Embryonic Stem Cell 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 Constitutive 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 Constitutive GFP Reporter Mouse Embryonic Stem Cells: (Catalog No. SCC082) 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 Constitutive GFP Reporter Mouse Embryonic Stem Cells have been validated for high level constitutive expression of GFP, 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). Cells display normal karyotype as assessed by G-banding of twenty metaphase cells and 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 Constitutive GFP Reporter Mouse Embryonic Stem Cells (Part No. SCC082) 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 Constitutive 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 Constitutive 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 Constitutive GFP Reporter Mouse Embryonic Stem Cells (Catalog No. SCC082)

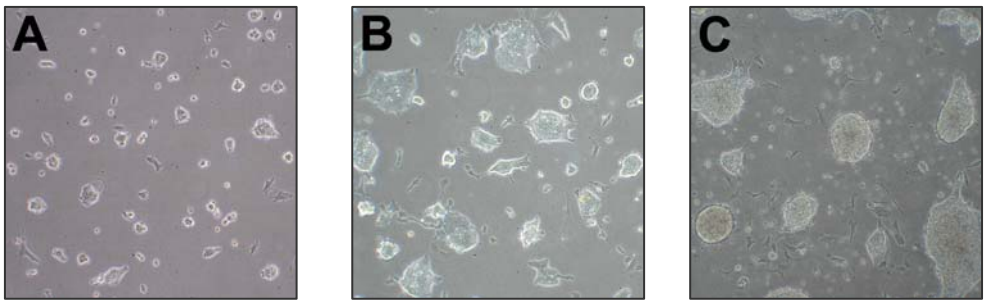


**Figure 1**. Phase contrast images of MilliTrace Constitutive GFP Reporter Mouse Embryonic Stem Cells (Catalog No. SCC082) after one (**A**) and two (**B**) days after thawing and also right before passaging (**C**). Cells were cultured on 0.1% gelatin coated 10-cm tissue culture plates in the absence of mouse feeder cells.

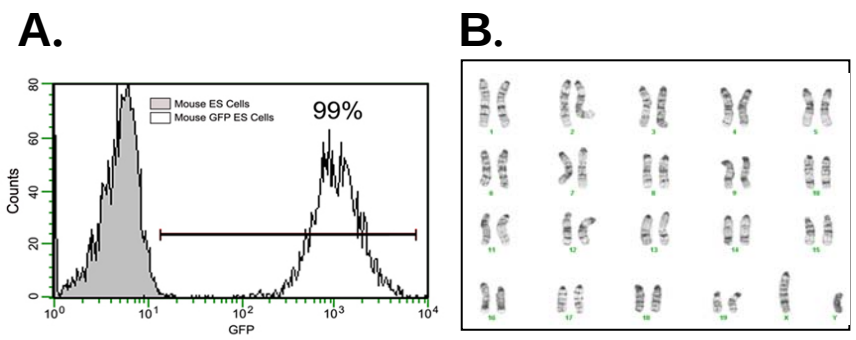


**Figure 2**. FACS analysis of MilliTrace Constitutive GFP Reporter Mouse Embryonic Stem Cells using a Guava flow cytometer indicates that >95% express GFP (**A**) and possess an apparently normal karyotype (**B**). Cytogenetic analysis was performed by Cell Line Genetics on twenty G-banded metaphase cells. Nineteen cells demonstrated an apparently normal male karyotype, while one cell demonstrated a non-clonal chromosome aberration which was attributed to a technical artifact. No abnormal cells with trisomy 8 and/or 11 were detected (**B**).

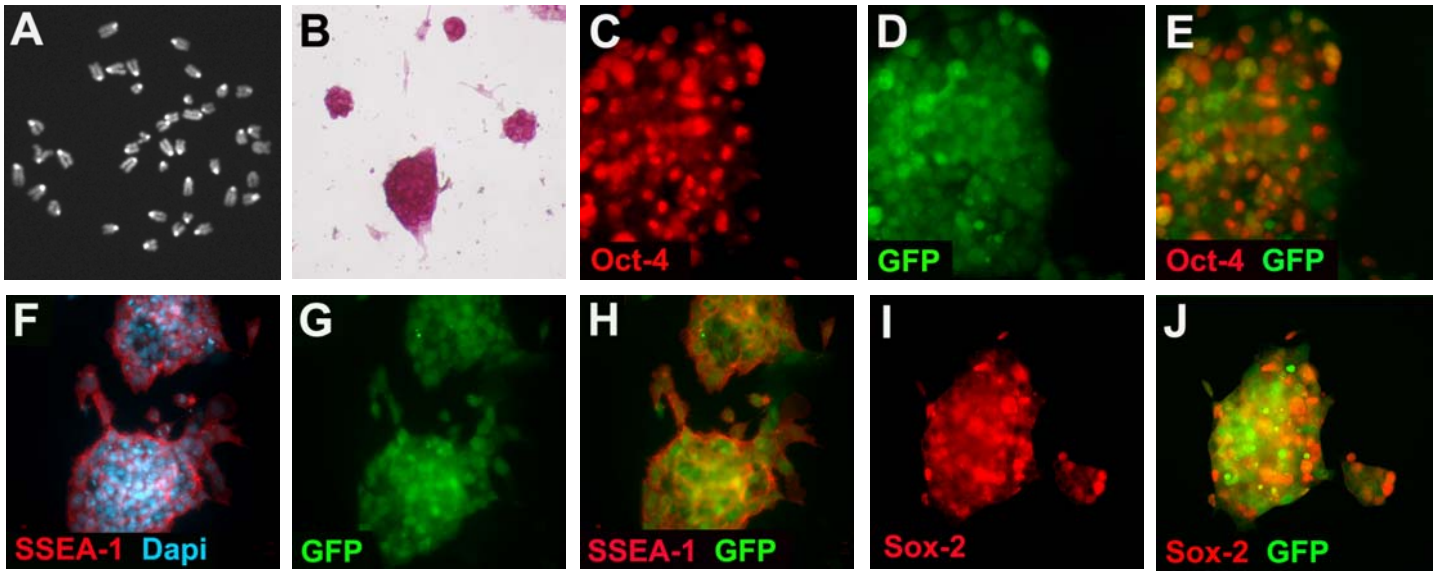


**Figure 3**. MilliTrace Constitutive GFP Reporter Mouse Embryonic Stem Cells (Catalog No. SCC082) contain the normal number of chromosomes (**A**) and express pluripotent ESC markers, alkaline phosphatase (**B**), OCT-4 (**C, E),** SSEA-1 (**F, H**) and Sox-2 (**I, J**). Nuclei of the cells were visualized with DAPI (**F**, blue). The OCT-4 and Sox-2 transcription factors are co-localized with the GFP staining in the nucleus (**E, J**). Majority of cells are GFP-positive (**D, G, and J**).

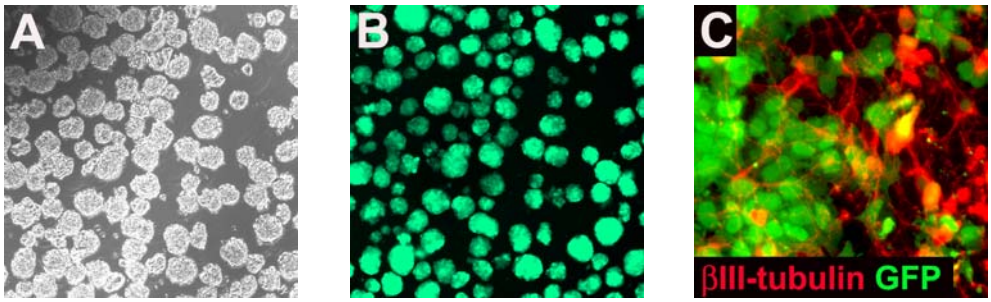


**Figure 4**. Constitutive GFP Reporter Mouse Embryonic Stem Cells are pluripotent. Using Mouse Embryonic Stem Cell Neurogenesis Kit (Catalog No. SCR101), ESC can differentiate into neurons (□III-tubulin, **C**, red). Phase contrast (A) and fluorescent (B) images of mouse embryoid bodies, respectively. All of the EBs are GFP-positive (**A, B**).

*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. EmbryoMax Complete ES Cell Media w/ 15%FBS and LIF: (Catalog No. ES-101-B)
4. Embryoid Body (EB) Formation Medium: (Catalog No. SCM018)
5. Laminin, mouse: (Catalog No. CC095)
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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