Fluorescent Mouse ES/iPS Cell Characterization Kit

Catalog No. SCR077

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

USA & Canada Phone: +1(800) 437-7500 Fax: +1 (951) 676-9209
Australia +61 3 9839 2000
www.millipore.com

Introduction

Mouse embryonic stem (mES) cells are pluripotent cells derived from the inner cell mass of pre-implantation blastocysts and are capable of unlimited, undifferentiated proliferation in vitro [1]. Mouse induced pluripotent (miPS) cells are pluripotent cells generated by reprogramming mouse somatic cells using four transcription factors, Oct-4, Klf-4, Sox-2, and c-Myc, or their variants[2]. Both mESC and miPSC can self-renew and have the ability to generate all three germ layers; ectoderm, mesoderm, and endoderm. Undifferentiated mouse ES/iPS cells can be maintained long term in media containing the cytokine, leukemia-inhibitory factor (LIF) or EMD Millipore's proprietary ES cell culture reagent, ESGRO[3-4]. However, upon removal of LIF from the culture medium, mouse ES/iPS cells start to differentiate into cells derived from all three germ layers. Several pluripotent markers are commonly used to distinguish pluripotent mESC/iPSC from differentiated cells.

- **Alkaline phosphatase (AP)** is an enzyme that hydrolyzes the phosphate group from many types of molecules, including nucleotides, proteins and alkaloids. Although AP is primarily found in liver and bone, pluripotent stem cells have also been found to have elevated expression of AP[5]. Both human and mouse ESC/iPSC are characterized by high expression levels of AP.
- **Oct-4 and Sox-2** are two transcription factors that are highly expressed in pluripotent cells. They share a significant proportion of their target genes and form the core transcriptional regulatory circuitry that contributes to pluripotency and self-renewal of mESC/iPSC[6]. The successful reprogramming of somatic cells with Oct-4, Sox-2 together with Klf-4 and c-Myc genes further confirms the essential role of these transcription factors in maintaining pluripotency[2, 7, 8].
- **SSEA-1** is a cell surface antigen that is expressed in pluripotent mouse ES/iPS cells and not on human ES/iPS cells[9].
- **DPPA-2** is a novel Oct-4 related protein that has binding sites for both POU and Sox-2 protein domains. These associations suggest a strong role for DPPA-2 in maintaining cell pluripotentiality[10].
- **DAPI** or 4', 6-diamidino-2-phenylindole is a fluorescent dye that binds strongly to A-T rich regions in DNA and is thus frequently used to label the cell nucleus.

EMD Millipore's Fluorescent Mouse ES/iPS Cell Characterization Kit contains a range of sensitive tools for the phenotypic assessment of the pluripotent status of mouse ES/iPS cells. Included in the kit is an enzymatic assay to measure alkaline phosphatase activity in the cells along with validated directly conjugated antibodies to proteins, Oct-4, Sox-2 and DPPA-2 that are critical to maintaining cell pluripotency along with the cell surface epitope SSEA-1 to enable rapid immunocytochemical marker analysis. The DAPI nuclear dye is conveniently included to aid in cell quantification.

For Research Use Only; Not for use in diagnostic procedures

Kit Components

1. Fast Red Violet solution (Part No. 90239): One 15 mL bottle.

2. Napthol AS-BI phosphate solution (Part No. 90234). One 15 mL bottle.

3. Mouse anti-Oct-4 (POU5F1), clone 7F9.2, Alexa Fluor® 488 conjugate (Part No. MAB4419A4-50UL). One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody.

4. Mouse anti-Sox-2, clone 10H9.1, Cy3 conjugate (Part No. MAB4423C3-50UL). One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody.

5. Mouse anti-SSEA-1, clone MC-480, Cy3 conjugate (Part No. MAB4301C3-50UL). One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody.

6. Mouse anti-DPPA-2, clone 6C1.2, Alexa Fluor® 488 conjugate (Part No. MAB4356A4-50UL). One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody.

7. DAPI, 100 µL (Part No. 90229). One vial containing 100 µL volume.

Related Products

The following related products are available from EMD Millipore as separate items.

1. Alkaline Phosphatase Detection Kit (Cat. No. SCR004).

2. Quantitative Alkaline Phosphatase ES Characterization Kit (Cat. No. SCR066)

3. Anti-OCT-4 [POU5F1], clone 7F9.2, Alexa Fluor® 488 conjugate, 100 µL (Cat. No. MAB4419A4)

4. Anti-OCT-4 [POU5F1], clone 7F9.2, Cy3 conjugate, 100 µL (Cat. No. MAB4419C3)

5. Anti-OCT-4, clone 10H11.2, Alexa Fluor® 488 conjugate, 100 µl (Cat No. MAB4401A4)

6. Anti-OCT-4, clone 10H11.2, Cy3 conjugate, 100 µl (Cat No. MAB4401C3)

7. Anti-NANOG, clone 7F7.1, Alexa Fluor® 488 conjugate, 100 µL (Cat. No. MABD24A4)

8. Anti-NANOG, clone 7F7.1, Cy3 conjugate, 100 µL (Cat. No. MABD24C3)

9. Anti-Sox-2, clone 10H9.1, Cy3 conjugate, 100 µL (Cat. No. MAB4423C3)

10. Anti-Sox-2, clone 10H9.1, Alexa Fluor® 488 conjugate, 100 µL (Cat. No. MAB4423A4)

11. Anti-SSEA-1, clone MC-480, Cy3 conjugate, 100 µL (Cat. No. MAB4301C3)

12. Anti-TRA-1-60, clone TRA-1-60, Cy3 conjugate, 100 µL (Cat. No. MAB4360C3)

13. Anti-TRA-1-60, clone TRA-1-60, Alexa Fluor® 488 conjugate, 100 µL (Cat. No. MAB4360A4)

14. Anti-TRA-1-81, clone TRA-1-81, Cy3 conjugate, 100 µL (Cat. No. MAB4381C3)

15. Anti-TRA-1-81, clone TRA-1-81, Alexa Fluor® 488 conjugate conjugate, 100 µL (Cat. No. MAB4381A4)

16. Anti-DPPA-2, clone 6C1.2, Alexa Fluor® 488 conjugate, 100 µL (Cat. No. MAB4356A4)

17. Anti-DPPA-2, clone 6C1.2, Cy3 conjugate, 100 µL (Cat. No. MAB4356C3)

Materials Not Supplied

1. Tissue culture-wares and supplies

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

3. Millicell EZ SLIDE 8-well glass, sterile (Cat. No. PEZGS0896)

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

5. 1X Rinse Buffer (e.g. TBST: 20 mM Tris-HCL, pH 7.4, 0.15M NaCl, 0.05% Tween 20)

6. Blocking Solution (3% normal goat or donkey serum, 0.2% Triton X-100, 0.05% NaN_3 in 1X PBS)

7. Non-Permeable Blocking Solution (3% normal goat or donkey serum in 1X PBS).

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

9. Microscope

Storage

The Fluorescent Mouse ES/iPS Cell Characterization Kit contains two components used for alkaline phosphatase activity determination as well as 4 ES cell-specific antibodies and a nuclear staining dye. When stored at 2° to 8℃, the kit components a re good for 4 months from date of receipt. Do not freeze or expose to elevated temperatures.

Preparation of Reagents

1. Naphthol/Fast Red Violet Solution: Mix Fast Red Violet (FRV) with Naphthol AS-BI phosphate solution and water in a 2:1:1 ratio (FRV:Naphthol:water) fresh before each staining assay.

Staining Protocol

Alkaline Phosphatase Staining Procedure:

1. Culture mouse ES/iPS cells for three to five days prior to analyzing AP activity. (NOTE: This time-period is critical to be able to observe good levels of AP activity).

2. Aspirate the media and fix the mouse ES/iPS cells with a fixative (e.g. 4% paraformaldehyde in 1X PBS) for 1-2 minutes.

   Note: *Do not overfix. Fixing cells longer than 2 minutes will result in the inactivation of alkaline phosphatase.*

3. Aspirate the fixative and rinse with 1X Rinse Buffer. DO NOT allow the cells to dry.

4. Prepare reagents for Alkaline Phosphatase staining as described in "Preparation of Reagents" section.

5. Add enough stain solution to cover each well (e.g. 2 mL for a well of a 6-well plate). Incubate in the dark at room temperature for 15 minutes.

6. Aspirate the staining solution and rinse the wells with 1X Rinse Buffer. Cover the cells with 1X PBS to prevent drying and then count the number of colonies expressing AP (red stem cell colonies), versus the number of differentiated colonies (colorless).

7. AP staining criteria: Greater than 90% of colonies should remain undifferentiated and express alkaline phosphatase.

Immunofluorescent Staining Procedure:

For optimal results, cell staining should be performed on cell colonies that have been in culture for approximately 3-5 days after passaging.

1. Culture mouse ES or iPS cells in a 24-well plate in mouse ESC expansion media of choice (0.5 ml volume per well). The staining protocol will work similarly using feeder or feeder-free media systems so please follow the manufacturer's instructions for specific media.

2. Remove the media from the wells. Be careful to not aspirate the cells.

3. Rinse once with 1X PBS then aspirate.

4. Add 4% Paraformaldehyde (PFA, diluted in 1X PBS) to each well. Incubate for 15-30 minutes at room temperature.

5. Carefully aspirate the PFA from the wells. Be careful to not aspirate the cells.

6. Wash three times with 1X PBS (~2-3 minutes per wash). At this point the fixed cells can be stored in 1X PBS at 4℃ for a couple of weeks if ne cessary.

7. Aspirate the 1X PBS. Apply a blocking solution for 30 – 60 minutes at room temperature or overnight at 4℃. **IMPORTANT: Do not shake the cells**. For optimal results, use the Blocking

Solution (3% Normal Goat or Donkey Serum, 0.2% Triton X-100, and 0.05% NaN_3 in 1X PBS) with antibodies directed against intracellular gene targets, Oct-4, Sox-2, and DPPA-2. Use the Non-Permeable Blocking Solution (3% Normal Goat or Donkey Serum in 1X PBS) with antibodies directed against the cell surface epitope, SSEA-1.

8. Before the end of the incubation time, prepare 1:100 dilutions of the conjugated antibodies in the appropriate blocking buffer (protected from light).

9. Aspirate the blocking buffer. Be careful to not aspirate the cells.

10. Add the 1:100 diluted antibodies to the designated well(s). Incubate for 1-2 hours at room temperature. Cover the plate(s) with tin foil to protect from the light.

11. Aspirate to remove the antibodies. Be careful to not aspirate the cells.

12. Wash three times with 1X PBS (3-4 minutes per wash).

13. Prepare the DAPI dye. Dilute the DAPI in 1X PBS at 1:1000 dilution.

14. Remove the last wash, add DAPI staining solution and incubate at room temperature for 5-10 minutes.

15. Remove the DAPI solution; wash three times with 1X PBS (3-4 minutes per wash).

16. If cell staining is on plates, cells should be covered with 1X PBS for visualization. However, if using glass coverslips, mount the coverslip onto glass slides using anti-fading mounting solution (e.g. DABCO/PVA).

17. Visualize the cell staining using a fluorescence microscope.
Note: Be sure to use the correct filter when visualizing fluorescent-labeled cells.

Results

The following are representative results obtained by using the Fluorescent Mouse ES/iPS Cell Characterization Kit on pluripotent mouse ESCs and iPSCs.

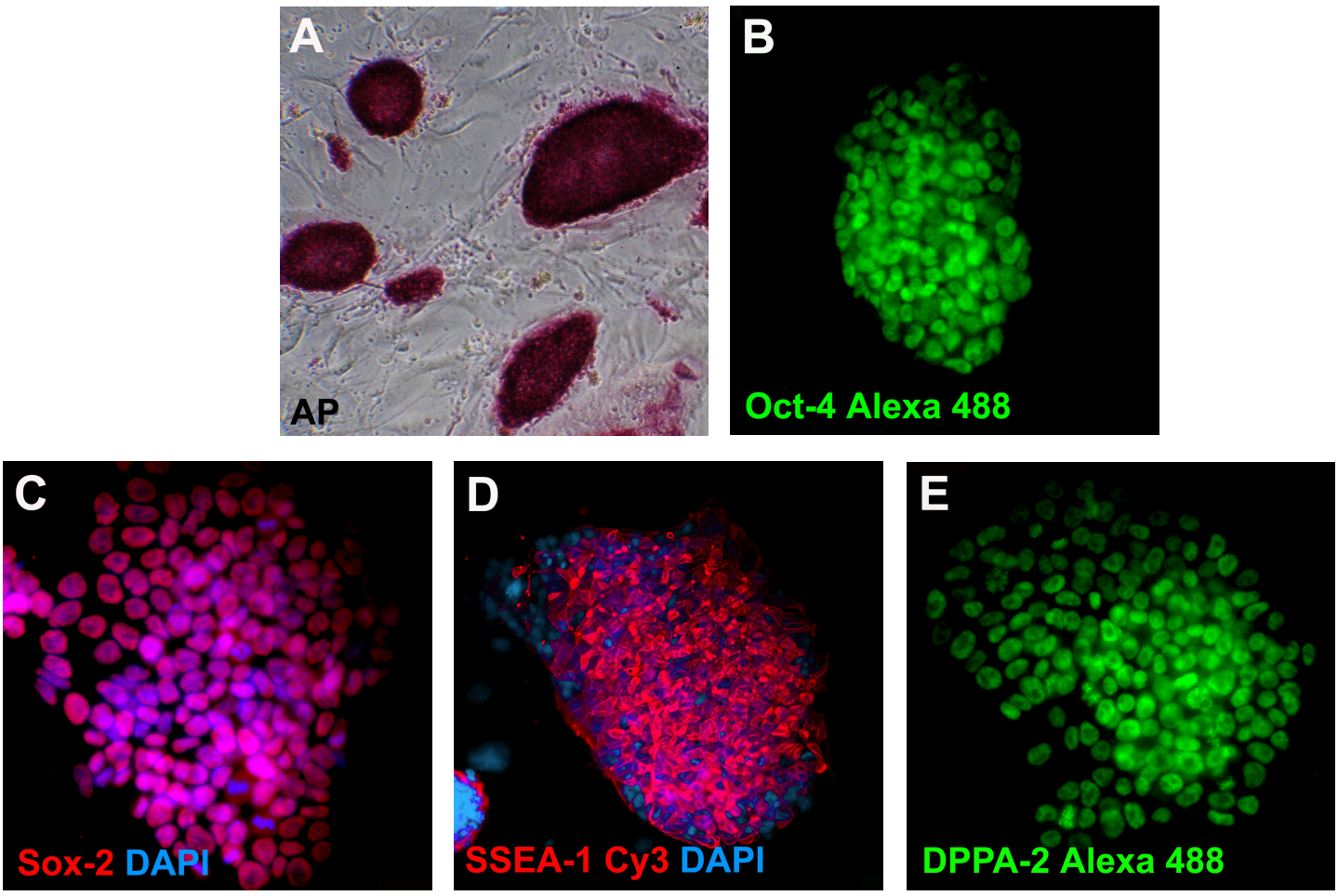

Figure 1. Pluripotent mouse ES/iPS cells express pluripotent markers, alkaline phosphatase (40x) (**A**), Oct-4 Alexa 488 (400x) (**B**), Sox-2 Cy3 (400x) (**C**), SSEA-1 Cy3 (200x) (**D**), and DPPA-2 Alexa 488 (400x) (**E**). All conjugated antibodies were used at 1:100 dilutions. Nuclei were counterstained with DAPI (blue). Mouse embryonic fibroblasts (p3) were reprogrammed using the STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (Cat. No. SCR510).

References

1. Evans, M. J. and Kaufman, M. H. (1981) Establishment in culture of pluripotential cells from mouse embryos. *Nature* **292(5819)**: 154-6.

2. Takahashi, K. and Yamanaka, S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **126(4)**: 663-76.

3. Smith, A. G, Heath, J. K., Donaldson, D. D., Wong, G. G., Moreau, J., Stahl, M., and Rogers, D. (1988) Inhibition of pluripotential embryonic stem cell differentiation by purified polypeptides. *Nature* **336(6200)**: 688-90.

4. Williams, R. L., Hilton, D. J., Pease, S., Wilson, T. A., Stewart, C. L., Gearing D, P., Wagner, E. F., Metcalf, D., Nicola, N. A., and Gough, N. M. (1988) Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* **336(6200)**: 684-7.

5. Pease, S., Braghetta, P., Gearing, D., Grail, D., Williams, R. L. (1990) Isolation of embryonic stem (ES) cells in media supplemented with recombinant leukemia inhibitory factor (LIF). *Dev. Biol* **141(2)**: 344-52.

6. Chen, L., and Daley, G. Q. (2008) Molecular basis of pluripotency. *Hum. Mol. Gene*t **17(R1)**: R23-7.

7. Nichols, J, Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Scholer, H., and Smith, A. (1998) Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4. *Cell* **95(3)**: 379-91.

8. Avillion, A. A., Nicolis, S. K., Pevny, L. H., Perez, L., Vivian, N., and Lovell-Badge, R. (2003) Multipotent cell lineages in early mouse development depend on SOX2 function. *Genes Dev*. **17(1)**: 126-40.

9. Solter, D. and Knowles, B. B. (1978) Monoclonal antibody defining a stage-specific mouse embryonic antigen (SSEA-1). *Proc. Natl. Acad. Sci. U. S. A* **75(11)**: 5565-9.

10. Maldonado-Saldivia, J., van den Bergen, J., Krouskos, M., Gilchrist, M., Lee, C., Li, R., Sinclair, A. H., Surani, M. A., and Western, P. S (2007) Dppa2 and Dppa4 are closely linked SAP motif genes restricted to pluripotent cells and the germ line. *Stem Cells* **25(1)**: 19-28.

Warranty

EMD Millipore Corporation (“EMD Millipore”) warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for a period of one year from shipment of the products. **EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications and descriptions of EMD Millipore products appearing in EMD Millipore’s published catalogues and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, EMD Millipore Corporation’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore Corporation promptly of any such breach. If after exercising reasonable efforts, EMD Millipore Corporation is unable to repair or replace the product or part, then EDM Millipore shall refund to the Company all monies paid for such applicable Product. **EMD MILLIPORE CORPORATION SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

Alexa Fluor is a registered trademark of Life Technologies. Cy3 is a registered trademark of GE Healthcare.)