User Manual

Fluorescent Mouse ES/iPS Cell Characterization Kit

SCR077

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

Product Overview

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 our proprietary ES cell culture reagent, ESGRO3-4. 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 AP5. 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/iPSC6. 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.

Our 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.

Materials Provided

- Fast Red Violet solution (90239): One 15 mL bottle
- Napthol AS-BI phosphate solution (2 mg/mL) in a buffer, pH 8.5 (CS235583): One 15 mL bottle
- Mouse anti-Oct-4 (POU5F1), clone 7F9.2, Alexa Fluor® 488 conjugate (MAB4419A4-50UL): One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody
- Mouse anti-Sox-2, clone 10H9.1, Cy3 conjugate (MAB4423C3-50UL): One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody
- Mouse anti-SSEA-1, clone MC-480, Cy3 conjugate (MAB4301C3-50UL): One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody
- Mouse anti-DPPA-2, clone 6C1.2, Alexa Fluor® 488 conjugate (MAB4356A4-50UL): One vial containing 50 µL of 0.5 mg/mL conjugated monoclonal antibody
- DAPI, 100 µL (90229): One vial containing 100 µL volume

Materials Required (Not supplied)

- Tissue culture-wares and supplies
- Fixative (e.g., 4% Paraformaldehyde in 1X PBS)
- Millicell® EZ SLIDE 8-well glass, sterile (PEZGS0896)
- Phosphate-Buffered Saline (1X PBS) (BSS-1005-B)
- 1X Rinse Buffer (e.g., TBST: 20 mM Tris-HCL, pH 7.4, 0.15 M NaCl, 0.05% Tween®-20)
- Blocking Solution (3% normal goat or donkey serum, 0.2% Triton™ X-100, 0.05% NaN_3 in 1X PBS)
- Non-Permeable Blocking Solution (3% normal goat or donkey serum in 1X PBS)
- Anti-fading mounting solution (DABCO/PVA)
- Microscope

Storage and Stability

See Materials Provided for 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 2-8 °C, the kit components are good for 4 months from date of receipt. Do not freeze or expose to elevated temperatures.

Protocol

Preparation of Reagents

Naphthol/Fast Red Violet Solution

Mix Fast Red Violet (FRV) with Naphthol AS-BI phosphate solution (2 mg/mL) in a buffer, pH 8.5 in a 1:1 ratio (FRV:Naphthol) 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).

   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 °C for a couple of weeks if necessary.
7. Aspirate the 1X PBS. Apply a blocking solution for 30-60 minutes at room temperature or overnight at 4 °C.

   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.

Product Performance

Results

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

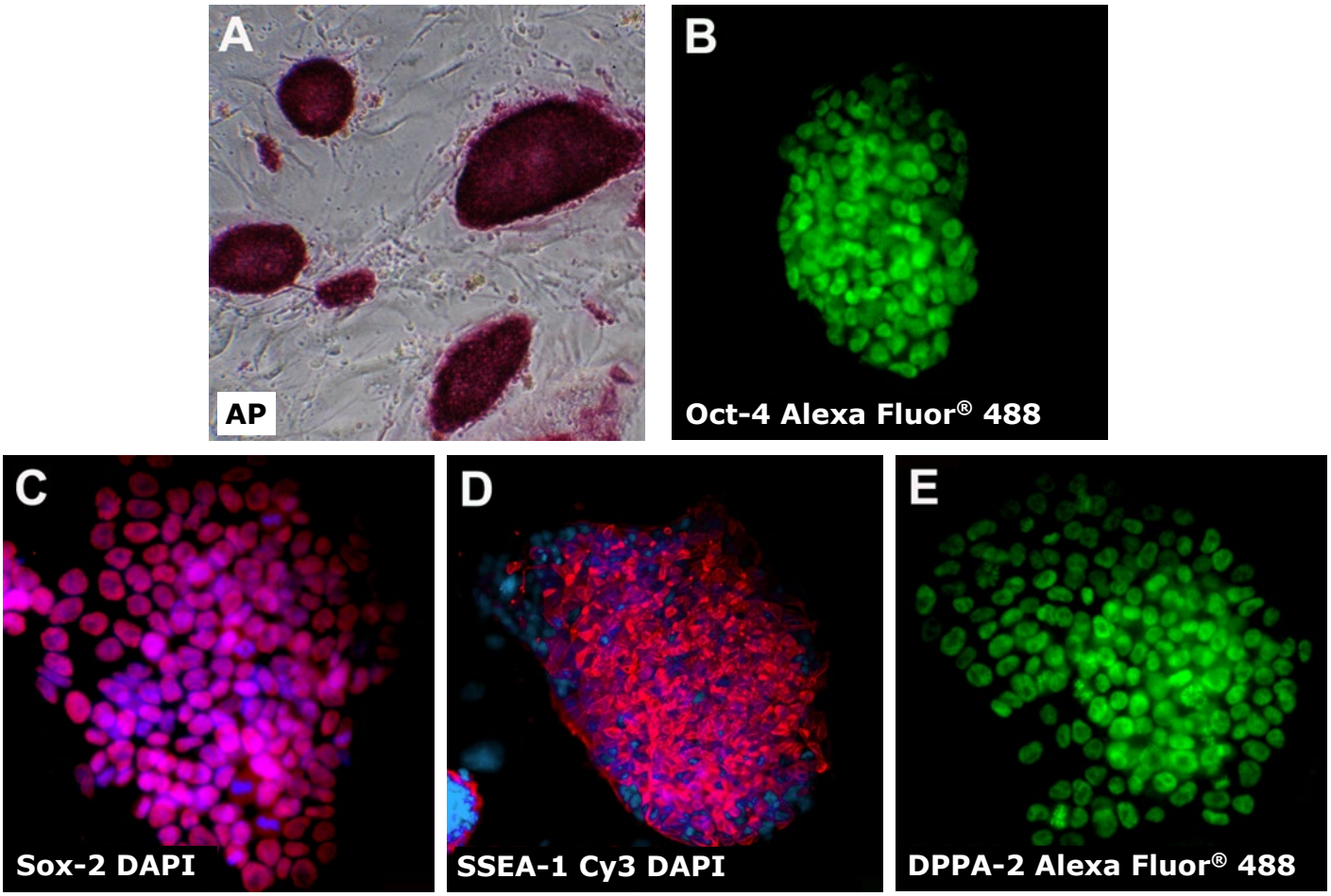

Figure 1. (**A**) Pluripotent mouse ES/iPS cells express pluripotent markers, alkaline phosphatase (40x), (**B**) Oct-4 Alexa Fluor® 488 (400x), (**C**) Sox-2 Cy3 (400x), (**D**) SSEA-1 Cy3 (200x), and (**E**) DPPA-2 Alexa Fluor® 488 (400x). All conjugated antibodies were used at 1:100 dilutions. Nuclei were counterstained with DAPI (blue). Mouse embryonic fibroblasts (p3) were reprogrammed using the STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit (SCR510).

Related Products

The following related products are available from SigmaAldrich.com as separate items.

- Alkaline Phosphatase Detection Kit (SCR004)
- Quantitative Alkaline Phosphatase ES Characterization Kit (SCR066)
- Anti-OCT-4 [POU5F1], clone 7F9.2, Alexa Fluor® 488 conjugate, 100 µL (MAB4419A4)
- Anti-OCT-4 [POU5F1], clone 7F9.2, Cy3 conjugate, 100 µL (MAB4419C3)
- Anti-OCT-4, clone 10H11.2, Alexa Fluor® 488 conjugate, 100 µL (MAB4401A4)
- Anti-OCT-4, clone 10H11.2, Cy3 conjugate, 100 µL (MAB4401C3)
- Anti-NANOG, clone 7F7.1, Alexa Fluor® 488 conjugate, 100 µL (MABD24A4)
- Anti-NANOG, clone 7F7.1, Cy3 conjugate, 100 µL (MABD24C3)
- Anti-Sox-2, clone 10H9.1, Cy3 conjugate, 100 µL (MAB4423C3)
- Anti-Sox-2, clone 10H9.1, Alexa Fluor® 488 conjugate, 100 µL (MAB4423A4)
- Anti-SSEA-1, clone MC-480, Cy3 conjugate, 100 µL (MAB4301C3)
- Anti-TRA-1-60, clone TRA-1-60, Cy3 conjugate, 100 µL (MAB4360C3)
- Anti-TRA-1-60, clone TRA-1-60, Alexa Fluor® 488 conjugate, 100 µL (MAB4360A4)
- Anti-TRA-1-81, clone TRA-1-81, Cy3 conjugate, 100 µL (MAB4381C3)
- Anti-TRA-1-81, clone TRA-1-81, Alexa Fluor® 488 conjugate, 100 µL (MAB4381A4)
- Anti-DPPA-2, clone 6C1.2, Alexa Fluor® 488 conjugate, 100 µL (MAB4356A4)
- Anti-DPPA-2, clone 6C1.2, Cy3 conjugate, 100 µL (MAB4356C3)

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