ES Cell Characterization Kit

For 100 Tests

Catalog No. SCR001

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Introduction

Stem cells have become the subject of extensive investigation recently, partly due to their therapeutic potential and because they raise several fundamental issues concerning the regulation of proliferation and differentiation.

Embryonic stem (ES) cells are derived from totipotent cells of the early mammalian embryo and are capable of unlimited, undifferentiated proliferation *in vitro* [1, 2, 3]. Undifferentiated mouse ES cells can be maintained for a long time in media containing the cytokine, leukemia-inhibitory factor (LIF) or Millipore's proprietary ES cell culture reagent, ESGRO® [4, 5]. However, upon removal of LIF from the culture medium, *in* vitro, the mouse ES cells start to differentiate into cells derived from all three germ layers. In contrast, human ES cultures require mouse fibroblasts as feeder cells and cannot be maintained with LIF for self-renewal [6, 7].

The undifferentiated state of the embryonic stem cell is characterized by high level of expression of alkaline phosphatase (AP) [8] and the stem cell transcription factor, Oct-4. Nevertheless, these ES cells also exhibit marked differences from their murine counterparts in regards to their expression of stage-specific embryonic antigen (SSEA), that typify undifferentiated human ES and embryonic carcinoma (EC) cells.

SSEA-1, a carbohydrate antigen, is a fucosylated derivative of type 2 polylactosamine and appears during late cleavage stages of mouse embryos. It is strongly expressed by undifferentiated, murine ES cells [9, 10]. Upon differentiation, murine ES cells are characterized by the loss of SSEA-1 expression and may be accompanied, in some instances, by the appearance of SSEA-3 and SSEA-4 [11]. In contrast, human ES and EC cells typically express SSEA-3 and SSEA-4 but not SSEA-1, while their differentiation is characterized by downregulation of SSEA-3 and SSEA-4 and an upregulation of SSEA-1 [12, 13]. Undifferentiated, human ES cells also express the keratin sulphate-associated antigens, TRA-1-60 and TRA-1-81 [14].

MILLIPORE's ES Cell Characterization Kit (Catalog number SCR001) is a specific and sensitive tool for the phenotypic assessment of the differentiation status of ES cells by measuring their AP activity, cell-surface stage-specific antigens (SSEA-1, SSEA-4) as well as expression of TRA-1-60, TRA-1-81 antigens.

Related Products

The following related products are available from MILLIPORE as separate items:

1. SSEA-1 Monoclonal Antibody, purified 100µg (Catalog # MAB4301)
2. SSEA-3 Monoclonal Antibody, purified 100µg (Catalog # MAB4303)
3. SSEA-4 Monoclonal Antibody, purified 100µg (Catalog # MAB4304)
4. TRA-1-60 Monoclonal Antibody, purified 100µg (Catalog # MAB4360)
5. TRA-1-81 Monoclonal Antibody, purified 100µg (Catalog # MAB4381)
6. Murine LIF, 5µg (Catalog # LIF2005)
7. Murine LIF, 10µg (Catalog # LIF2010)
8. ESGRO®, 1 x 10^6 units (Catalog # ESG1106)
9. ESGRO®, 1 x 10^7 units (Catalog # ESG1107)

Product Description and Storage

The ES Cell Characterization Kit consists of two components used for alkaline phosphatase activity determination as well as four ES cell-specific antibodies required to perform 100 tests (including controls).

When stored at 2º to 8ºC, the kit components are stable up to the expiration date. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the expiration date.

Kit Components

1. Fast Red Violet solution (0.8g/L stock) (Part No. 90239). Two 15mL bottles.
2. Napthol AS-BI phosphate solution (4mg/mL) in AMPD buffer (2mol/L), pH 9.5 (Part No. 90234). One 15mL bottle.
3. MS X SSEA-1, IgM, clone MC-480 (Part No. 90230). One vial containing 100µl of 1mg/mL monoclonal antibody.
4. MS X SSEA-4, IgG, clone MC-813-70 (Part No. 90231). One vial containing 100µl of 1mg/mL monoclonal antibody.
5. MS X TRA-1-60, IgM, clone TRA-1-60 (Part No. 90232). One vial containing 100µl of 1mg/mL monoclonal antibody
6. MS X TRA-1-81, IgM, clone TRA-1-81 (Part No: 90233). One vial containing 100µl of 1mg/mL monoclonal antibody.

Materials Required But Not Supplied

1. Fixative (e.g. 4% Paraformaldehyde)
2. 1 x Rinse Buffer (e.g. TBST: 20mM Tris-HCl, pH 7.4, 0.15 NaCl, 0.05% Tween-20)
3. Blocking solution
4. Fluorescent-labeled secondary antibodies
5. Hematoxylin
6. Microscope

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).

Antibody Specificity

	SSEA-1	SSEA-4	TRA-1-60	TRA-1-81
Human ES cell	✗	✓	✓	✓
Murine ES cell	✓	✗	✗	✗

Legends:

✗ = undifferentiated ES cells show negative immunostaining for this antibody

✓ = undifferentiated ES cells show positive immunostaining for this antibody

Staining Protocol

A. *Alkaline Phosphatase Staining Procedure*

1. Culture ES cells for five days prior to analyzing AP activity, at low to medium density (*NOTE: This time-period is critical if activity levels of AP needs to be observed. According to our findings, five days of culturing are optimal for good AP stain visualization).*

2. On day five, aspirate media and fix the ES or EC cells with a fixative (*e.g.* 4% Paraformaldehyde in 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 fixative and rinse with 1 X Rinse Buffer. DO NOT allow wells 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. 0.5mL for a well of a 24-well plate). Incubate in dark at room temperature for 15 minutes.
6. Aspirate staining solution and rinse wells with 1 X Rinse Buffer. Cover cells with 1 X 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 in the well containing 10^3 Units of LIF. P value shall be ≥ 0.05.

B. Immunofluorescence Staining Procedure

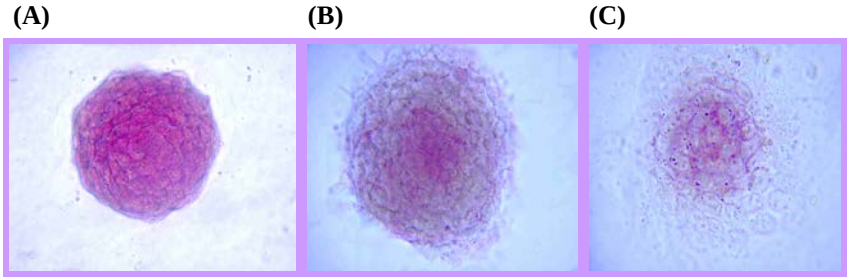
The cell-surface-antigen expression of cultured cells can be analyzed by using immunofluorescence techniques. The following primary monoclonal antibodies are used to detect surface-antigen expression: anti-SSEA-1; anti-SSEA-4; TRA-1-60 and TRA-1-81 (provided in kit). Fluorescein isothiocyanate (FITC)-labeled goat anti-mouse IgM or anti-IgG can be used as secondary antibody, appropriate to the isotype of the primary antibody.

1. Fix cultured ES cells in 4% paraformaldehyde/PBS for 15-20 minutes at room temperature.
2. Wash twice (5-10 minutes each) with 1 X Rinse Buffer.
3. Permeabilize cells with 0.1% Triton X-100/PBS for 10 minutes at room temperature.
4. Wash twice (5-10 minutes each) with 1X Rinse Buffer.
5. Apply a Blocking solution (e.g. 4% normal goat serum/PBS) for 30 minutes at room temperature.
6. Dilute primary antibodies to working concentrations, in blocking solution (SSEA-1, SSEA-4, TRA-1-60 and TRA-1-81 can be used in the range of 1:10 – 1:50). Incubate primary antibodies for 1 hour at room temperature.
7. Wash three times (5-10 minutes each) with 1X Rinse Buffer.
8. Dilute secondary antibodies in 1 X PBS just before use. Incubate secondary antibodies for 30-60 minutes at room temperature.

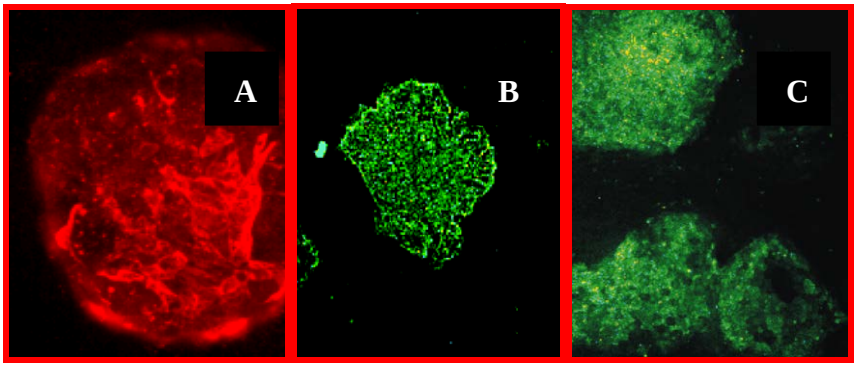
9. Wash three times (5-10 minutes each) with 1X Rinse Buffer.
10. If stained in plate wells, cells should be covered with 1X PBS for visualization. However, if cells are stained on a coverslip, mount on a slide by using antifade mounting solution.
11. Fluorescence images can be visualized with a fluorescence microscope.

    Note: *Be sure to use the correct filtervisualizing fluorescently labeled cells.*

Staining results obtained with ES Cell Characterization Kit



Alkaline Phosphatase staining of ES cells. High magnification revealed **(A)** Undifferentiated ES cells (mouse MBL.5 cell line) – cultured for five days in media containing MILLIPORE's LIF/ESGRO®. A concentration of 10^3 Units/mL is used for inhibition of differentiation. **(B)** Differentiated ES cells – cultured at low-medium density for three days in media without any LIF/ESGRO®. **(C)** Differentiated ES cells – cultured at low-medium density for six days in media without any LIF/ESGRO®.



Immunofluorescence images of ES cells, stained with anti-SSEA and anti-TRA antibodies. (A) undifferentiated, murine ES cells (cultured in presence of

LIF) revealed positive immunostaining for SSEA-1. **(B)** Immunofluorescence staining of human ES cell colony with anti-SSEA-4 monoclonal antibody. As anticipated, ES cells within the colony exhibit strong immunoreactivity to this antibody. **(C)** Immunofluorescence staining of human ES cells with anti-TRA-1-60 monoclonal antibody. As revealed by the image, the ES cells within the colony exhibit strong immunoreactivity to this antibody.

Photographs B and C courtesy of Dr. Jonathan Draper, University of Sheffield, Department of Biomedical Science.

References

1. Evans M, Kaufman M. *Nature* **292**, 154 (1981).
2. Martin G. *Proc Natl Acad Sci USA* **78**, 7634 (1981).
3. Morrison SJ, Shah NM, Anderson DJ. *Cell* **88**, 287-298 (1997).
4. Smith AG, Heath JK et al. *Nature* **336**, 688-690 (1988).
5. Williams RL, Hilton DJ et al. *Nature* **366**, 684-687 (1988).
6. Thomson JA, Marshall VS. *Curr Top Dev Biol* **38**, 133 (1998).
7. Shamblott MJ, Axelman J et al. *Proc Natl Acad Sci USA* **95**, 13726-13731 (1998).
8. Pease S, Braghetta P et al. *Develop Biol* **141**, 344 (1990).
9. Solter D, Knowles BB. *Proc Natl Acad Sci USA* **75**, 5565-5569 (1978).
10. Gooi HC, Feizi T, Kapadia A et al. *Nature* **292**, 156-158 (1981).
11. Solter D, Shevinsky LH, Knowles BB. *Dev Biol* **70**, 515-521 (1979).
12. Andrews PW. *Dev Biol* **103**, 285-293 (1984).
13. Fenderson BA, Andrews PW et al. *Dev Biol* **122**, 21-34 (1987).
14. Andrews PW, Banting GS et al. *Hybridoma* **3**, 347-361 (1984).

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