Alkaline Phosphatase Detection Kit

For 100 Tests

Catalog No. SCR004

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Application

Embryonic stem (ES) cells are totipotent cells derived from the inner cell mass (ICM) of preimplantation mammalian embryos and are capable of unlimited, undifferentiated proliferation *in vitro* [1,2,3]. Undifferentiated murine ES cells can be maintained *in vitro* for extended periods in media containing the cytokine, leukemia-inhibitory factor (LIF) or MILLIPORE®'s proprietary ES cell culture reagent, ESGRO® [4,5]. The undifferentiated state of ES cells can be characterized by high level of expression of Alkaline Phosphatase (AP) [6], the expression of surface markers including SSEA and TRA antigens and the transcription factor Oct-4.

MILLIPORE®'s Alkaline Phosphatase Detection Kit (Catalog number SCR004) is a specific and sensitive tool for the phenotypic assessment of ES cell differentiation by the determination of AP activity.

Available separately from MILLIPORE® are the monoclonal antibodies TRA-2-49 (Catalog number MAB4349) and TRA-2-54 (Catalog number MAB4354), which permit the detection of Liver / Bone / Kidney isozyme of AP as expressed by ES cells. When used in flow cytometry, these reagents permit a quantitative assessment of AP expression by ES cells [7].

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Related Products

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

1. ES Cell Characterization Kit (Catalog # SCR001)
2. ES Marker Sample Kit (Catalog # SCR002)
3. ES Cell 3D Collagen Culture Kit (Catalog # SCR003)
4. SSEA-1 Monoclonal Antibody, purified 100μg (Catalog # MAB4301)
5. SSEA-3 Monoclonal Antibody, purified 100μg (Catalog # MAB4303)
6. SSEA-4 Monoclonal Antibody, purified 100μg (Catalog # MAB4304)
7. TRA-1-60 Monoclonal Antibody, purified 100μg (Catalog# MAB4360)
8. TRA-1-81 Monoclonal Antibody, purified 100μg (Catalog# MAB4381)
9. TRA-2-49 Monoclonal Antibody, purified 100μg (Catalog # MAB4349)
10. TRA-2-54 Monoclonal Antibody, purified 100μg (Catalog # MAB4354)
11. Murine LIF, 5μg (Catalog # LIF2005), 10μg (Catalog # LIF2010)
12. ESGRO®, 1 x 10^6 units (Catalog # ESG1106), 1 x 10^7 units (Catalog # ESG1107)

Storage

The Alkaline Phosphatase Detection Kit consists of two components used for determining AP activity. 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. Naphthol AS-BI phosphate solution (4mg/mL) in AMPD buffer (2mol/L), pH 9.5 (Part No. 90234). One 15mL bottle.

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, 0.15M NaCl, 0.05% Tween-20)
3. Hematoxylin (optional)
4. 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).

Staining Protocol

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 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.

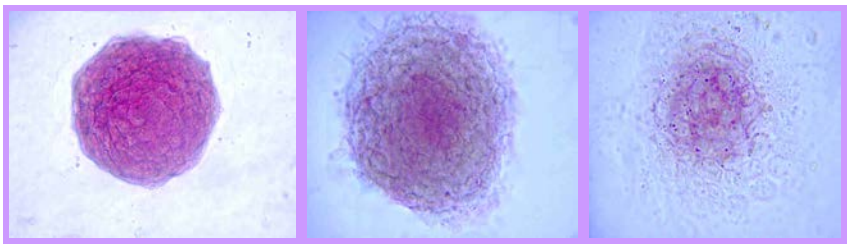
5. Add enough stain solution to cover each well (*e.g*. 0.5mL for a well of a 24-well plate). Incubate in dark at room temperature for 15 minutes.

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.

Staining with Alkaline Phosphatase Detection Kit

(A) **(B)** **(C)**



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®.

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. Pease S, Braghetta P et al. *Develop Biol* **141**, 344 (1990).

7. Draper J, et al. *J.Anat.* **200**, 249 - 258 (2002)

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