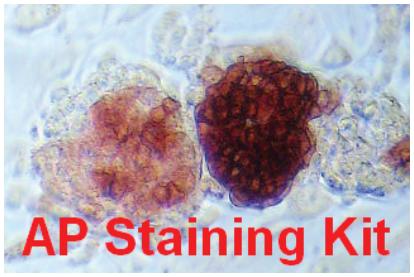

AP Staining Kit

Cat. # AP100B-1
Cat. # AP100R-1
Cat. # AP100D-1

User Manual

Store 4 °C on receipt

A limited-use label license covers this product. By use of this product, you accept the terms and conditions outlined in the Licensing and Warranty Statement contained in this user manual.

(ver. 1-101910)

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I. Introduction and Background

A. Overview

Alkaline phosphatase (AP) is a universal pluripotent marker for all types of pluripotent stem cells including embryonic stem cells, embryonic germ cells, and induced pluripotent stem cells. The pluripotent status of stem cells can be characterized by a high level of AP expression, along with the expression of multiple pluripotency markers including the transcription factors Nanog, Oct4, Sox2, stage-specific embryonic antigens, SSEA-1, -3, -4, and tumor related antigens, TRA-1-60, TRA-1-81.

Cell-type	AP	SSEA-1	SSEA-4	TRA-1-60	TRA-1-81
Mouse ES Cell	√	√	--	--	--
Mouse EG Cell	√	√	--	--	--
Human ES Cell	√	--	√	√	√
Human EG Cell	√	√	√	√	√
Human EC Cell	√	--	√	√	√

ES cell = Embryonic stem cell
EG cell = Embryonic germ cell
EC cell = Embryonic carcinoma cell

The Dual-Color AP staining kit is a specific, sensitive, and reliable tool for phenotypic assessment of AP expression at the surface of stem cells. The Dual-Color AP kit provides sufficient reagents for a total of 100 assays (24-well plate formate) with both Red and Blue (50 assays for each color). The AP Red (50 assays) or AP Blue kits (50 assays) are also sold separately.

Alkaline phosphatase (AP) is a hydrolase enzyme responsible for dephosphorylating molecules such as nucleotides and proteins under alkaline conditions. The Dual-Color Alkaline Phosphatase Staining Kit is a histochemical assay for use on cells grown in tissue culture wells or dishes. It uses a solution containing substrate and color dye as the colorimetric read-out for the enzymatic activity. The AP-positive, undifferentiated stem cells

stain red or blue. The stain develops quickly, does not readily fade, and can be analyzed 15 to 30 minutes after application to the cultured cells. For added convenience, the stained samples can be kept at 4^{O}C for up to one week for analysis.

B. Suggested Usage

- Measure alkaline phosphatase activities *in situ* (Immunohistochemistry)
- Quality control of stem cells
- Monitor cell differentiation/ pluripotency status

C. Workflow of AP Staining

Step 1: Seed stem cells in culture (Day 1)

Step 2: Perform experiment (Day 3-5)

Step 3: AP Staining (total time: ~2 hours)

1) Prepare Staining Solution

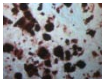
2) Wash twice with 1X PBS

3) Fix cells for 2-5 minutes

4) Wash twice with 1X PBS

5) Incubate for 15-20 min in the dark at room temperature

6) Stop reaction by washing twice with 1X PBS

7) Data analysis  

D. Kit Components

Blue- Color™ AP Staining Kit (Cat. # AP100 B-1):

Fixation Solution:	25 ml
Solution A Blue:	10 ml
Solution B Blue:	10 ml

Red- Color™ AP Staining Kit (Cat. # AP100 R-1):

Fixation Solution:	25 ml
Solution A Red:	10 ml
Solution B Red:	10 ml

Dual- Color™ AP Staining Kit (Cat. #AP100 D-1):

Fixation Solution:	25 ml X 2
Solution A Blue:	10 ml
Solution B Blue:	10 ml
Solution A Red:	10 ml
Solution B Red:	10 ml

E. Materials Needed but Not Supplied

- Human/mouse embryonic stem cells or induced pluripotent stem cells
- 1X PBS
- Light Microscope

F. Storage

Store all components at 4°C until their expiration dates.

II. AP Staining Protocol

A. Preparation of Reagents

Prepare sufficient AP substrate solution for each experiment. Quantities can be scaled up using a 1:1 ratio (by volume) of Solution A and Solution B. For optimal results, the AP substrate solution should be used within 30 minutes of preparation. The AP substrate solution can remain at room temperature afterwards.

B. Alkaline Phosphatase Staining

1. Culture mouse/human stem cells on feeder layer or feeder-free.

2. Gently aspirate the culture medium and wash the cells with 1 ml of 1X PBS. Aspirate the wash solution.

3. Add Fix Solution to the cells, 0.5 ml per well for a 24-well plate. Incubate at room temperature for 2 to 5 min. (Do not fix your cells beyond 20 min, as over-fixation may result in the loss of AP activity.)

4. Aspirate the Fix Solution and wash the fixed cells with 1X PBS twice.

5. Remove the 1X PBS and add freshly prepared AP Substrate Solution into each well. For a 24-well plate, add 0.4 ml per well.

6. Incubate the cells with substrate at room temperature for 15 to 20 minutes, protected from light.

7. Stop the reaction by aspirating the staining Solution and rinsing the wells twice with 1X PBS.

8. Cover the cells with 1X PBS to prevent drying out.

9. AP expression will result in a red/purple stain for Color Red and a blue stain for Color Blue, while the absence of AP expression will result in no stain.

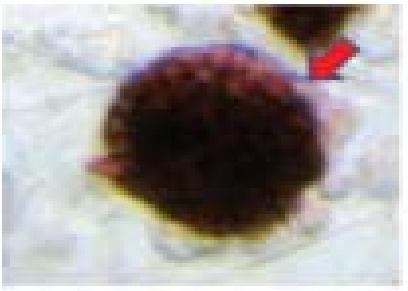
10. Observe the red/blue stained cell colonies (undifferentiated stem cells) vs. colorless colonies (differentiated stem cells or non-stem cells) using a light microscope.

Store the plate at 4^0C for future analysis (up to one week).

C. Example of Results

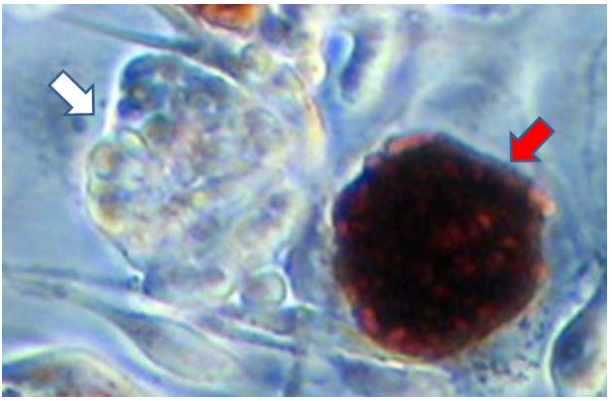
The following figures demonstrate typical results with the Dual-Color AP staining kit. Staining of mouse induced pluripotent stem cells using the Blue-Color and Red-Color AP staining kit. AP-positive colonies appear either blue or red to purple (indicated by blue or red arrow), while the surrounding feeder cells appear colorless.

Blue-Color Kit



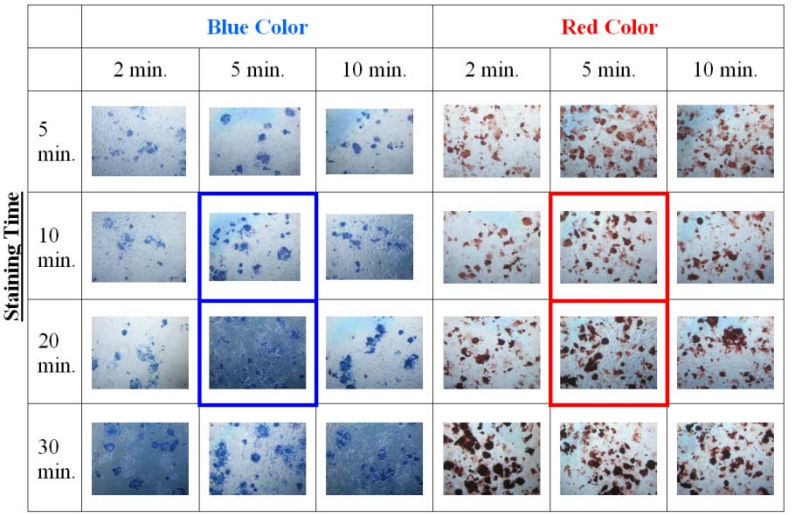
Red-Color Kit

Use the AP staining kit to discriminate between pluripotent versus differentiated cells as shown below. Staining of mouse induced pluripotent stem cells using the Red-Color AP staining kit. The differentiated colony (indicated by white arrow) appears colorless and undifferentiated colony (indicated by red arrow) appears red or purple.



AP Staining Picture Grid:

Fixation Time



III. Related Products

Pluripotency reporters
http://systembio.com/stem-cell-research/pluripotency-reporters/#catalog_listings

Differentiation reporters
http://systembio.com/stem-cell-research/differentiation-reporters/#catalog_listings

High quality feeder cells and source cells, and Human/ Mouse iPS cell lines
http://systembio.com/viral-ipscs/#viral-ipscs_tab_1_5

IV. References

1. Takahashi K, Yamanaka S. (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663-76.
2. Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., Yamanaka, S. (2007) Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131, 861-872.
3. Yu J., Vodyanik M.A., Smuga-Otto K., Antosiewicz-Bourget J., Frane J.L., Tian S., Nie J., Jonsdottir G.A., Ruotti V., Stewart R., Slukvin I.I., Thomson J.A. (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318, 1917-1920.

V. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

VI. Licensing and Warranty Statement

Limited Use License

Use of the Dual- Color™ AP Staining Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

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Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.