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# Alkaline Phosphatase Staining Kit

Cat # AP100B-1 and AP100P-1

## User Manual

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**Storage:**

Store at +4°C upon receipt

Version 6  
7/20/2021

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



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## Product Description

### Easily visualize undifferentiated cells with alkaline phosphatase stains

When you need to know which cells are undifferentiated, such as for monitoring pluripotency status, performing quality control of stem cells, or measuring alkaline phosphatase (AP) activity *in situ*, SBI's AP Staining Kits are specific, sensitive, and reliable tools that can provide answers in as little as fifteen minutes. Unlike other pluripotency markers that are only present in certain subsets of undifferentiated cells, AP is highly active in embryonic stem cells, embryonic germ cells, and induced pluripotent stem cells (Table 1), making it a universal pluripotency marker.

**Table 1. AP is a universal pluripotency marker**

Cell-type	AP	SSEA-1	SSEA-4	TRA-1-60	TRA-1-81
Mouse embryonic stem cell	✓	✓	--	--	--
Mouse embryonic germ cell	✓	✓	--	--	--
Human embryonic stem cell	✓	--	✓	✓	✓
Human embryonic germ cell	✓	✓	✓	✓	✓
Human embryonic carcinoma cell	✓	--	✓	✓	✓

- Obtain answers in as little as fifteen minutes
- Stain cells grown in dishes or the wells of tissue culture plates
- Get a long-lasting colorimetric readout with this quick and easy histochemical assay
- Use to monitor pluripotency status/differentiation, ensure the quality of stem cells, and measure AP activity *in situ*

Both Blue-Color and Purple-color Alkaline Phosphatase Staining Kits employ a histochemical assay that can be used on cells grown in dishes or the wells of tissue culture plates, and each kit includes substrate and dye for colorimetric read-out of enzymatic activity. The AP-positive, undifferentiated stem cells stain either purple (Cat.# AP100P-1) or blue (Cat.# AP100B-1), with color developing rapidly. The stain does not fade and stained samples can be kept at 4°C for up to one week.

**Table 2. AP Staining Kits**

Catalog number	Description	Application
<b>AP100P-1</b>	Purple-Color AP Staining Kit	Newest version for most applications
<b>AP100B-1</b>	Blue-Color AP Staining Kit	Legacy product for existing users who need continuity

### The easy AP staining workflow

Once your cells are ready, the AP staining workflow can be rapidly completed:

1. Wash cells in 1x PBS
2. Fix cells with Fix Solution (2 – 5 minutes)
3. Wash cells with 1x PBS
4. Stain cells with AP Stain in the dark (15 – 20 minutes)
5. Wash cells with 1x PBS to stop staining
6. Image cells and analyze

## List of Components

**Table 2. Components for the AP Staining Kits**

Catalog #	Product Name	Components	Volume	Storage Temperature
AP100P-1	Purple-Color AP Staining Kit	Fixation Solution	25 mL	4°C
		Solution Purple Staining	20 mL	4°C
AP100B-1	Blue-Color AP Staining Kit	Fixation Solution	25 mL	-4°C
		Solution A Blue	10 mL	4°C
		Solution B Blue	10 mL	4°C

## Additional Required and Optional Equipment Not Included in Kit

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

## Protocol

### For Purple-Color AP Staining Kit, for each well of a 24-well plate

**NOTE:** Unlike the older Blue-Color AP Staining Kit, the stain in the Purple-Color AP Staining Kit—**Solution Purple Staining**—comes pre-mixed and ready to use.

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 1X PBS. Aspirate the wash solution.

3. Add **Fixation Solution** to the cells, 0.25 mL per well for a 24-well plate. Incubate at room temperature for 4 min. (Do not fix your cells beyond 20 minutes as over-fixation may result in the loss of AP activity.)
4. Aspirate the **Fixation Solution** and wash the fixed cells with 1X PBS once
5. Remove the 1X PBS and add 200  $\mu$ L of AP **Staining Purple Solution** and cover with paper.
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 Purple Solution** and rinsing the wells once with 1X PBS.
8. Cover the cells with 1X PBS to prevent drying out.
9. Cells that express AP will stain purple whereas cells that don't express AP will remain unstained.
10. Observe the purple stained cell colonies (undifferentiated stem cells) vs. colorless colonies (differentiated stem cells or non-stem cells) using a light microscope.
11. Image using BX780 Keyence 10X objective

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

#### For the Blue-Color AP Staining Kit, for each well of a 24-well plate

##### Before you start:

For the Blue-Color AP Staining kit, 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.

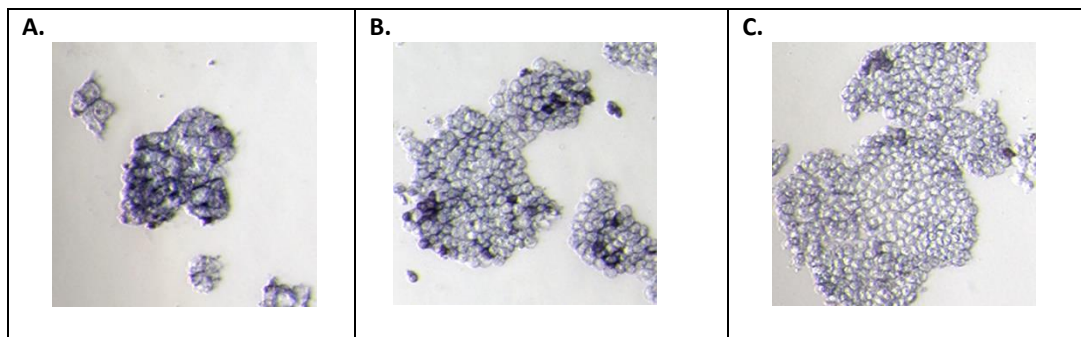
##### Blue-Color AP Staining protocol:

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 **Fixation Solution** to the cells, 0.25 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 **Fixation Solution** and wash the fixed cells with 1X PBS.
5. Remove the 1X PBS and add freshly prepared **AP Substrate Solution** into each well. For a 24-well plate, add 0.2 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 **AP Substrate Solution** and rinsing the wells with 1X PBS.
8. Cells that express AP will stain blue whereas cells that don't express AP will remain unstained.
9. Observe the 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°C for future analysis (up to one week).

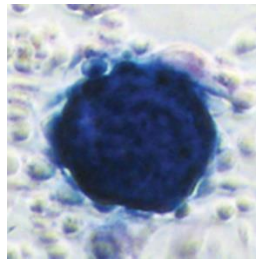
## Example Data and Applications

Visualize pluripotent cells with the Purple-Color AP Staining Kit



**Figure 1.** The Purple-Color AP Staining Kit delivers robust staining of pluripotent cells. Purple-Color stained (A) human teratocarcinoma cells, (B,C) passage 19 mouse teratocarcinoma cells.

Visualize pluripotent cells with the Blue-Color AP Staining Kit



**Figure 2.** With the Blue-Color AP Staining Kit, pluripotent cells stain blue while the surrounding feeder cells remain unstained.



## 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:

System Biosciences (SBI)  
2438 Embarcadero Way  
Palo Alto, CA 94303

**Phone:** (650) 968-2200  
**Toll-Free:** (888) 266-5066  
**Fax** (650) 968-2277

**E-mail:**  
General Information: [info@systembio.com](mailto:info@systembio.com)  
Technical Support: [tech@systembio.com](mailto:tech@systembio.com)  
Ordering Information: [orders@systembio.com](mailto:orders@systembio.com)

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System Biosciences (SBI)  
2438 Embarcadero Way  
Palo Alto, CA 94303

**Phone:** (650) 968-2200  
**Toll-Free:** (888) 266-5066  
**Fax:** (650) 968-2277

**E-mail:**

General Information: [info@systembio.com](mailto:info@systembio.com)  
Technical Support: [tech@systembio.com](mailto:tech@systembio.com)  
Ordering Information: [orders@systembio.com](mailto:orders@systembio.com)