

# Detection of AP using the Alkaline Phosphatase Staining Kit II

## Overview

The following procedure describes staining one well of a 6-well plate for Alkaline Phosphatase (AP) detection.

Product Description	Cat. No.	Format
REPROCELL Stemgent® Alkaline Phosphatase Staining Kit II	00-0055	500 rxn
Components	Size	Storage
Fix Solution	25 mL	4 °C
AP Staining Solution A	10 mL	4 °C
AP Staining Solution B	10 mL	4 °C
AP Staining Solution C	10 mL	4 °C

**Table 1:** REPROCELL Stemgent® Alkaline Phosphatase Staining Kit II contents.

For other plate formats, see the suggested amounts in Table 2.

## Additional Materials Required

- 1× PBS
- Tween® 20
- Mounting medium (optional)
- 15 mL conical tubes

## Material Preparation

- **PBST**  
In a 15 mL conical tube, add 10 mL of 1× PBS. Add 5 µL of Tween 20 for a final concentration of 0.05%. Mix well and store at room temperature.
- **AP Substrate Solution**  
For one well of a 6-well plate, mix 0.5 mL of Solution A and 0.5 mL of Solution B in a 15 mL conical tube. Incubate at room temperature for 2 minutes. Add 0.5 mL of Solution C.

*Note: Prepare only the amount of AP Substrate Solution necessary for each experiment. Quantities can be scaled up or down, as long as a 1:1:1 ratio is preserved. For optimal results, the AP Substrate Solution should be used within 30 minutes after preparation. Discard any remaining solution.*

## AP Staining of Cells

1. Aspirate the culture medium and wash the cells with 2 mL of 1× PBST.
2. Add 1 mL of Fix Solution and incubate at room temperature for 2 to 5 minutes.

*Note: Do not over fix the cells. Excessive fixation will result in the loss of AP activity.*

3. Aspirate the Fix Solution and wash the fixed cells with 2 mL of 1× PBST. Do not allow the wells to dry.
4. Aspirate the 1× PBST and add 1.5 mL of freshly prepared AP Substrate Solution.
5. Incubate the cells in the dark (wrapped with foil or in a dark container) at room temperature for 5 to 15 minutes.

*Note: Closely monitor the color change and stop the reaction when the color turns bright to avoid non-specific staining.*

6. Stop the reaction by aspirating the AP Substrate Solution and washing the wells twice with 2 mL of 1× PBS.
7. Cover the cells with 1× PBS or mounting medium to prevent drying.
8. AP expression will result in a red or purple stain, while the absence of AP expression will result in no stain.
9. Store the plate at 4 °C

Culture vessel	Surface area/well	Fix Solution	1× PBS	AP Staining Solution	Reactions Per kit
24-well plate	2 cm <sup>2</sup>	0.5 mL	0.5 mL	0.6 mL	50
12-well plate	3.8 cm <sup>2</sup>	1 mL	1 mL	1 mL	24
6-well plate	9.6 cm <sup>2</sup>	2 mL	2 mL	1.5 mL	12

Table 2. Suggested amounts per well.

Tween® is a registered trademark of ICI America, Inc

**REPROCELL USA Inc**

9000 Virginia Manor Road  
Suite 207  
Beltsville, MD 20705  
USA

Tel: +1 301 470 3362

Email: [info-us@reprocell.com](mailto:info-us@reprocell.com)

**REPROCELL Europe Ltd**

Thomson Pavilion, Todd Campus  
West of Scotland Science Park  
Acre Road  
Glasgow G20 0XA  
UK

Tel: +44 (0)141 465 3460

Email: [info-emea@reprocell.com](mailto:info-emea@reprocell.com)

**REPROCELL India Ltd**

3-1-135/1A, CNR Complex  
Mallapur  
Hyderabad 500 076  
Telangana  
India

Tel: +44 (0)141 465 3460

Email: [Bhargavi.Gurram@reprocell.com](mailto:Bhargavi.Gurram@reprocell.com)

**REPROCELL Inc (Japan)**

MetLife Shin-yokohama 381, Bldg. 9F  
3-8-11, Shin-yokohama Kohoku-ku,  
Yokohama  
Kanagawa 222-0033  
Japan

Tel: +81 45 475 3887

Email: [info-asia@reprocell.com](mailto:info-asia@reprocell.com)