Protocol

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. For other plate formats, see the suggested amounts in Table 1.

Product Description	Cat. No.	Format
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

ADDITIONAL MATERIALS REQUIRED

- 1X PBS
- Tween® 20
- Mounting medium (optional)
- 15 ml conical tubes

MATERIAL PREPARATION

- **PBST**

In a 15 ml conical tube, add 10 ml of 1X 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.*

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AP STAINING OF CELLS

1. Aspirate the culture medium and wash the cells with 2 ml of 1X 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 1X PBST. Do not allow the wells to dry.
4. Aspirate the 1X 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 1X PBS.
7. Cover the cells with 1X 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.

Table 1. Suggested Amounts per Well

Culture vessel	Surface area/well	Fix Solution	1X PBS	AP Staining Solution	Reactions Per kit
24-well plate	2 cm^2	0.5 ml	0.5 ml	0.6 ml	50
12-well plate	3.8 cm^2	1 ml	1 ml	1 ml	24
6-well plate	9.6 cm^2	2 ml	2 ml	1.5 ml	12

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