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^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

Table 2. Suggested amounts per well.

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