Quick Manual

AP-Juice (1 03 213/ 1 03 223)

Components included:

AP-Juice	**100ml** Buffer for measurement of Alkaline Phopshpatase. **Store at +4°C.**

Applications: Immunoassays, Reporter Gene assays, Quantitative PCR, Blotting (applicable on PVDF & Nitrocellulose membranes, a special product for Nylone membranes is available)

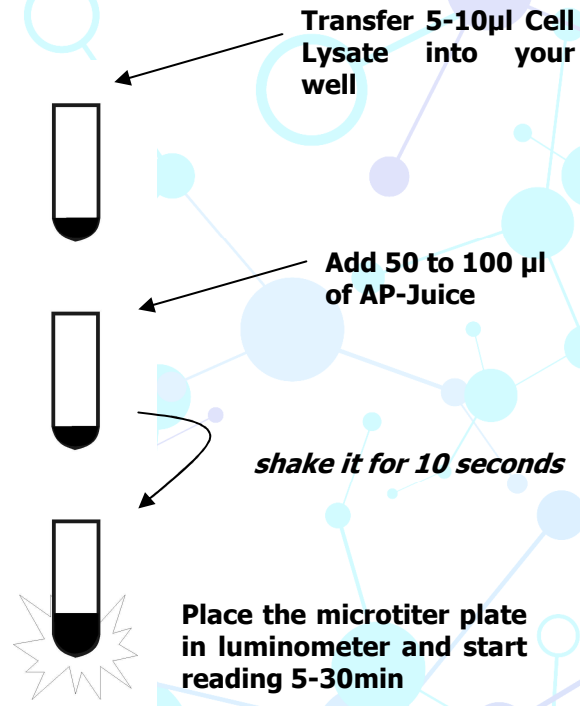
Standard Protocol for Detection of Alkaline Phosphatase in Microtiter Plate Luminometer

Equilibrate at room temperature for 30 minutes before use.
Note: *Do not contaminate the substrate with any phosphate buffer. Use only toxin free BSA (Sigma, A2934 or other source) as a blocking reagent for micro titer plates or membrane to lower the background luminescence.*

→ Quality of BSA can be checked by AP-Juice. Transfer 100 µl of AP-Juice in a tube or micro titer plate and record the background luminescence. Now add 10 µl of 0.1% BSA in tris buffer, pH 8.0 to 8.5, to 100 µl of AP-Juice and check the background luminescence. If background does not change, the quality of BSA is excellent.

- Add 5 to 10 µl of diluted cell extract to microplate wells.
- Add 50 to 100 µl of AP-Juice
- shake it for 10 seconds.
- Place the microtiter plate in luminometer and start reading after 5-30min.

Standard procedure:



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Quick Manual

AP-Juice (1 03 213/ 1 03 223)

Standard Protocol for Enzyme Immunoassay with AP

Note: *Do not contaminate the substrate with any phosphate buffer. Use only toxin free BSA (Sigma, A2934 or other source) as a blocking reagent for micro titer plates or membrane to lower the background luminescence.*

→ Quality of BSA can be checked by AP-Juice. Transfer 100 µl of AP-Juice in a tube or micro titer plate and record the background luminescence. Now add 10 µl of 0.1% BSA in tris buffer, pH 8.0 to 8.5, to 100 µl of AP-Juice and check the background luminescence. If background does not change, the quality of BSA is excellent.

Equilibrate at room temperature for 30 minutes before use.

Coating plates:

- Wash the plate with washing buffer
- Block the remaining active sites of the plate with blocking buffer (check the blocking buffer with AP substrate for background)
- Wash the plate with washing Coat microtiter plate with antibodies or antigene buffer and dry at room temperature or room temperature under vacuum and store properly

Assay for haptens (anti-hapten antibodies coated plates)

- Add the diluted antigen-enzyme conjugate to the plate with different concentration of antigen and incubate at room temperature or 37°C for 30 to 120 minutes
- Wash the plate with washing buffer at least three times (washing buffer should be 0,2M tris-buffer, pH 7,2 to 7,5)
- Add the AP-Juice to the plate and incubate at room temperature or 37°C for 30 to 120 minutes
- Read the plate

Assay for proteins (anti-protein antibodies coated plates):

- Add the different concentration of antigen protein diluted in buffer to the plate and incubate for 30 to 120 minutes
- Wash the plate three times with washing buffer
- Add the anti-protein antibody (monoclonal)-enzyme (AP) conjugate diluted in enzyme diluent buffer and incubate 30 to 120 minutes
- Wash the plate at least three times with washing buffer (for alkaline phosphatase washing buffer should be 0,2M tris-buffer pH 7,2 to 7,5)
- Add the AP-Juice and incubate at room temperature or 37°C for 30 to 120 minutes
- Read the plate

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