Alkaline Phosphatase (AP) Chemiluminescent Substrate for ELISA / Membrane Blotting

Product Description:

Alkaline Phosphatase (AP) Chemiluminescent Substrate for ELISA / Membrane Blotting (RB-APCS) can detect AP at extremely low levels in solution. RB-APCS is a stabilized 1, 2-dioxetane substrate. Recommended for blotting applications and detection of alkaline phosphatase labeled nucleotide and protein probes in solution and on solid supports such as membranes including ELISA, Western Blots and Southern blots or any other solid surface. The lower enzyme concentration detection can range from a few attograms to the highest concentration being a few nano-grams. RB-APCS is an ultra-sensitive substrate. RB-APCS chemiluminescent substrates provide superior sensitivity compared to competitor products and are more economical. The substrate is supplied as a single bottle reagent. The substrate can be used with various high-quality toxin free blocking buffers. Detection and analysis requires a chemiluminometer (for ELISA) or imaging system (for Blot).

Hazard Identification: Please see MSDS.

Product Stability, Storage and Specifications:

RB-APCS shelf life is 24 months when stored in the dark at 2° C to 8° C. Keep container tightly closed. Store RB-APCS away from heat and light.

Product Use:

ELISA DIRECTIONS

1. Use a clean container, aliquot an appropriate volume for use, and equilibrate the reagent to room temperature before use. For optimal results, wash your container and micro titer plate with 0.2M tris buffer, pH 9.5 to 9.7.
2. Do not contaminate the substrate with any phosphate buffer or extraneous sources of alkaline phosphatase or other proteins including bacterial derived AP. Never pipette directly from the RB-APCS stock bottle or pour aliquoted solution back into the stock vessel.
3. Analytes can be assayed in appropriate microtiter plates designed for chemiluminescence. Conditions should be optimized for each assay system.
4. RB-APCS is extremely sensitive. Optimal detection may be obtained from 15 minutes to 120 minutes after contacting the substrate with AP enzyme.

MEMBRANE DIRECTIONS

1. Store RB-APCS at 2-8°C. Equilibrate to room temperature before use. Do not contaminate the substrate with any phosphate buffer or extraneous sources of alkaline phosphatase or other proteins including bacterial derived AP. Never pipette directly from the RB-APCS stock bottle or pour aliquoted solution back into the stock vessel. Handle blots with clean gloves and clean forceps.

2. Analytes can be applied to membranes as a dot blot or via gel transfer. Blotting conditions should be optimized for each assay system. RB-APCS is extremely sensitive.

3. Block the membrane with blocking buffer. For Westerns, incubate the membrane with primary antibody using optimized concentrations and timing. Wash the membrane with wash buffer several

times. Incubate the membrane with AP conjugated probe using optimized concentrations and timing. Wash the membrane with wash buffer several times. Add RB-APCS to the membrane and incubate for 2 to 3 minutes. Use approximately 100 uL of RB-APCS per square centimeter of membrane. Remove excess substrate from the membrane.

4. Visualize by either x-ray film or a CCD imaging system. Peak signal may be obtained approximately 30 minutes after substrate contact which is maintained for several hours.