

Product Information

Rapid Chemi AP Substrate

Catalog Number **SAE0056**
Storage Temperature 2–8 °C

Synonym: Disodium 2-chloro-5-(4-methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)-1-phenyl phosphate

Product Description

Rapid Chemi AP Substrate is a sensitive, chemiluminescent substrate for alkaline phosphatase that allows for the rapid, reproducible detection of alkaline phosphatase-labeled molecules. Rapid Chemi AP Substrate is supplied as a ready-to-use aqueous solution, i.e., no dilution is necessary, for use in a variety of membrane-based applications such as Western blotting and ELISA. Other applications include Southern and Northern Blotting, detection of placental alkaline phosphatase, detection of secreted alkaline phosphatase, reporter gene assays, nucleic acid probe assays, and alkaline or acid phosphatase biomarker detection (stem cells and tumor markers).

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at 2–8 °C when not in use. Keep the substrate away from direct sunlight and bright artificial light. Remains active for at least one year when stored and handled in an aseptic manner.

Do not use diluents or wash buffers that contain EDTA or other chelators as they can remove divalent cations (Mg^{2+} , Zn^{2+}) and cause loss of AP activity.

After use, do not return excess reagent to the original bottle. Keep the excess in a separate container. Test saved excess reagent for activity and background before use in another assay.

Procedures

Notes: Avoid Contamination with Environmental Phosphatases - All alkaline phosphatase substrates can be easily contaminated with phosphatases present in the environment causing false-positive results and shortening the reagent shelf life. Take care to prevent contamination of the substrate solution. In addition, some common biochemical reagents, such as BSA may contain trace amounts of alkaline phosphatase activity. Test all reagents for alkaline phosphatase activity before use.

Optimization of Antibody Concentrations - When using chemiluminescent detection in immunologic assays, optimal results are often obtained when antibody concentrations are lower than those used for chromogenic detection. For best results, empirically determine the optimal antibody concentrations that generate the highest level of sensitivity

General Procedure for Western Blotting:

1. Let reagent bottle come to room temperature. Aliquot amount required into a new container prior to use. Do not pipette directly from the bottle.
2. Use 0.1 mL of Chemi AP per square centimeter of membrane.
3. Incubate the blot for 5 minutes in the Chemi AP substrate.
4. Remove blot from the Chemi AP substrate and drain excess liquid.
5. Place the blot in clear plastic wrap and remove bubbles.
6. Expose the blot to X-ray film or use an imaging system.
7. Adjust exposure time to obtain optimal results.

General Procedure for ELISA:

1. Let the Chemi AP reagent bottle come to room temperature.
2. For the signal generation step, wash the ELISA plate with a Tris-based buffer containing TWEEN[®] 20 or Triton[™] X-100.
3. Remove excess liquid from the plates.
4. Add 100 μ l of Chemi AP to each well.
5. Shake the plate for 20-60 seconds at 600-1,000 rpm.
6. Read the plate immediately or up to 60 minutes after the shaking has been completed. Signal will increase linearly for ~15 minutes. Signal will continue to increase for up to 90 minutes.
7. Adjust the luminometer gain, read time, and/or integration time to obtain optimal results.

Results

Figure 1.

Typical Signal Kinetics of Rapid Chemi AP Substrate

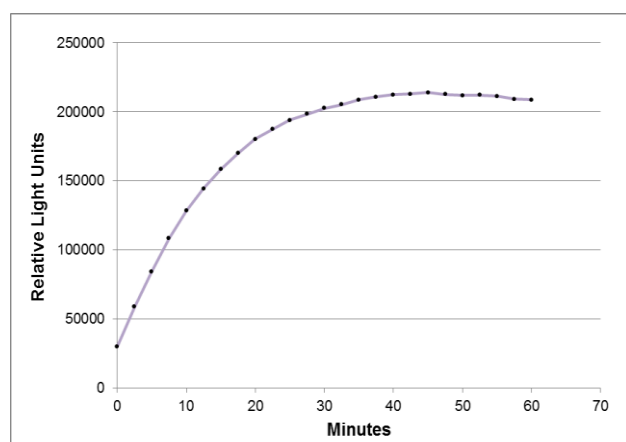
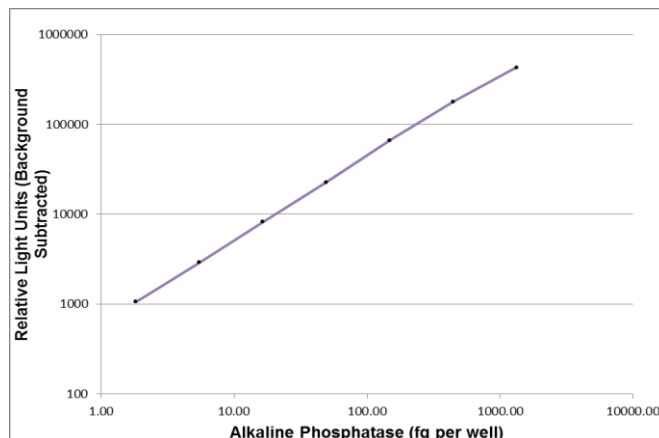


Figure 2.

Typical Alkaline Phosphatase Dose Response



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