

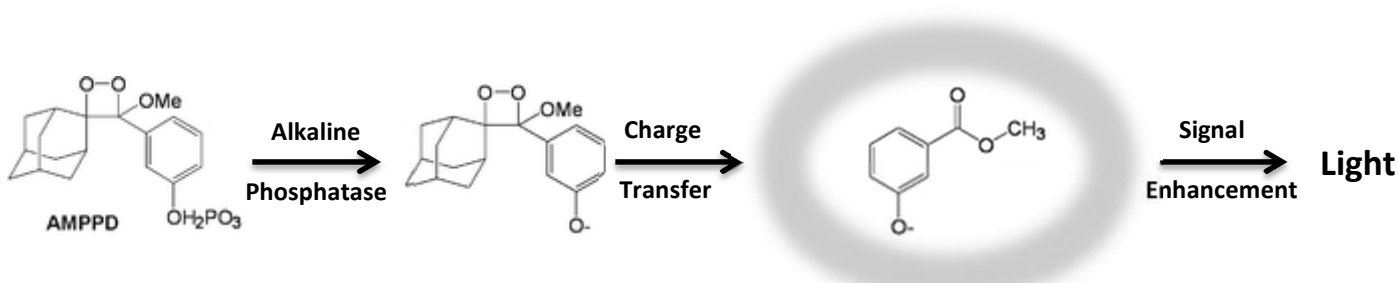
Moss Alk Phos Chemiluminescent Substrates

ChemiAP-RK & ChemiAP-MK

High-Sensitivity Single-Component Chemiluminescent Alkaline Phosphatase Substrates for ELISA and Western Blot

The Moss enhanced chemiluminescent substrate is a highly sensitive single-component reagent for the quantitative detection of Alkaline Phosphatase bound to a solid phase or in free solution. The substrate is stable at room temperature for up to one week at room temperature or for up to 12 months when refrigerated. The substrate yields a linear response with the concentrations of Alkaline Phosphatase commonly employed in immunologic assays.

The substrate is based on well-known 1,2-dioxetane chemistry but incorporates novel enhancers.



Light emission is initiated when alkaline phosphatase cleaves phosphate from AMPPD resulting in a highly energetic anion intermediate. After further degradation, the energetic anion transfers its energy to fluorescein which then emits the transferred energy as visible light. The efficiency of energy transfer and conversion to visible light is enhanced nearly 1000-fold by the proprietary enhancers. In addition, the kinetics of the reaction can be varied by varying the composition of the enhancers.

Because these substrates may be more sensitive than needed for some applications, an optional diluent is available that can be used to adjust the signal strength to a level consistent with the specific sensitivity and dynamic range requirements of a particular assay.

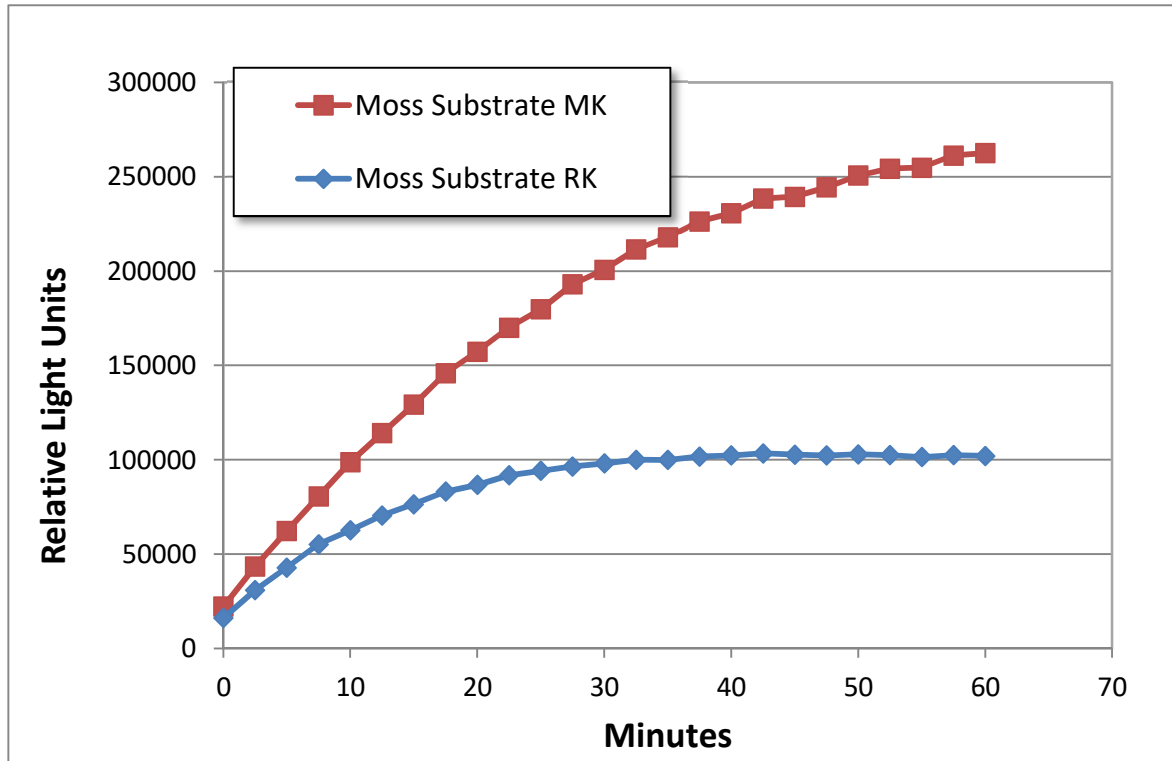
Products:

Catalog Number	Description	Volume
ChemiAP-RK	Ready-to-use Alkaline Phosphatase Chemiluminescent Substrate RK (Rapid Kinetics)	Each product is available in 100, 500 or 1000 mL sizes
ChemiAP-RKDil	Optional Diluent for Substrate RK	
ChemiAP-MK	Ready-to-use Alkaline Phosphatase Chemiluminescent Substrate MK (Maximum Kinetics)	
ChemiAP-MKDil	Optional Diluent for Substrate MK	



Difference between RK and MK Substrates

RK (Rapid Kinetic Substrate) has a strong initial activity and the signal reaches 90% of maximum after approximately 20 minutes. MK (Maximum Kinetic Substrate), a more powerful substrate, has a higher initial reaction rate than RK and produces a signal that reaches 90% of maximum after approximately 45 minutes. Both substrates will emit light for several hours. An example of the relative signal strength and reaction kinetics of both substrates is shown in the graph below (150 fg of calf intestinal alkaline phosphatase added to 100 µl of substrate).



Storage and Shelf Life:

- Store the product at 2-8°C.
- The product can be stored for up to one year at 2-8°C.

Important Information:

- Keep reagent bottles at 2-8°C when not in use.
- Allow bottles to warm to room temperature before opening.
- Aliquot amount required into a new container prior to use. Do not pipette directly from the bottle.
- 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.
- 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.
- Keep the substrate away from direct sunlight and bright artificial light.



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.

Note about 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 ELISA:

1. Let the ChemiAP 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 ChemiAP to each well.
5. Shake the plate for 20-60 seconds at 600-1000 rpm.
6. Read the plate immediately or up to 60 minutes after the shaking has been completed. Signal will increase linearly for approximately 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.

General Procedure for Western Blotting:

1. Let reagent bottle come to room temperature.
2. Use 0.1 mL ChemiAP per square centimeter of membrane.
3. Incubate the blot for 5 minutes in the ChemiAP substrate.
4. Remove blot from the ChemiAP 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.



Instructions for Use of the ChemiAP Diluent

ChemiAP Diluent can be used to dilute ChemiAP Substrate in order to reduce the intensity of the signal generated while also providing significant cost savings.

To obtain a lower signal output consistent with the sensitivity and dynamic range needs of your assay, consider testing the following dilutions of the ChemiAP Substrate with the ChemiAP Diluent:

% Strength	Preparation
50%	1 Part Substrate + 1 Part Diluent
40%	1 Part Substrate + 1.5 Parts Diluent
30%	1 Part Substrate + 2.33 Parts Diluent
20%	1 Part Substrate + 4 Parts Diluent
10%	1 Part Substrate + 9 Parts Diluent
5%	1 Part Substrate + 19 Parts Diluent

Certificate of Analysis (COA)

A lot-specific COA is available upon request. The COA provides detailed quality information for each specific lot.

Related Products:

Catalog Number	Description	Volume
ChemiHRP-100	2-Part Chemiluminescent Substrate for Horseradish Peroxidase	100, 500 or 1000 mL
ChemiHRP-A	Chemiluminescent Substrate for HRP, Part A (Enhanced Luminol Solution)	50, 250 or 500 mL
ChemiHRP-B	Chemiluminescent Substrate for HRP, Part B (Stable Peroxide Solution)	50, 250 or 500 mL
ChemiHRP-DIL	Optional Diluent for Chemiluminescent Substrate for HRP, Part A	100, 500 or 1000 mL

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