



Moss Chemiluminescent Substrate for HRP

Enhanced Chemiluminescent Substrate for ELISA and Western Blot

INTRODUCTION

The Moss enhanced chemiluminescent substrate is a highly sensitive two-component reagent for the quantitative detection of HRP bound to a solid phase or in free solution. The substrate is stable at room temperature for six months or for up to 36 months when refrigerated. The substrate yields a linear response with the concentrations of HRP commonly employed in immunologic assays.

When catalyzed by HRP, oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at a wavelength of 425 nm. The quantum yield of the non-enhanced reaction is quite low but has been significantly increased by the addition of proprietary enhancers. Light emission begins immediately upon mixing the substrate and HRP. At high levels of HRP, the maximum signal is achieved immediately, and the signal slowly decays over the course of several hours. At low levels of HRP, the signal reaches a maximum after about five minutes, plateaus for 30-60 minutes and then slowly decays.

When mixed and stored at room temperature in the dark or in a brown bottle, the working solution is stable for up to 24 hours and shows less than a 10% drop in signal and no increase in background. 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.

Product:

CHEMI-100: 100 mL (50 mL Part A and 50 mL Part B).

CHEMI-500: 500 mL (250 mL Part A and 250 mL Part B).

CHEMI-1000: 1000 mL (500 mL Part A and 500 mL Part B).

Components:

CHEMI-A: Chemiluminescent Substrate, Part A (Enhanced Luminol Solution)

CHEMI-B: Chemiluminescent Substrate, Part B (Stabilized Peroxide Solution)

Optional Component:

CHEMI-DIL: 1000 mL Part A Diluent

Dunn Labortechnik GmbH · Thelenberg 6 · 53567 Asbach

Tel. +49(0) 6 83 / 4 30 94 · Fax +49(0)26 83 / 4 27 76 ·

e-mail: info@dunnlab.de · Internet: www.dunnlab.de

Stability and Shelf Life:

The shelf life of the product is guaranteed for a minimum of 36 months at 2-8°C.

Important Information:

- Keep reagent bottles at 2-4°C when not in use.
- Do not use solutions that contain sodium azide because azide inhibits HRP activity.
- Do not allow any metallic object to contact the substrate. Metal ions can activate the substrate.
- The working solution is stable for 24 hours in the dark at room temperature.

Procedure for ELISA:

1. Let reagent bottles come to room temperature.
2. Prepare Working Solution by mixing equal parts of CHEMI-A and CHEMI- B.
3. The Working Solution is stable for 24 hours in the dark at room temperature.
4. Wash ELISA plate with a Phosphate or Tris-based buffer containing Tween-20 or Triton X-100.
5. Remove excess liquid from the plates.
6. Add 100 µl of Working Solution to each well.
7. Shake the plate for 30-60 seconds at 600-1000 rpm.
8. Read the plate 5-20 minutes after the shaking has been completed.
9. Adjust the luminometer gain and/or integration time to obtain optimal results.

Procedure for Western Blotting:

1. Let reagent bottles come to room temperature.
2. Prepare Working Solution by mixing equal parts of CHEMI-A and CHEMI-B.
3. The Working Solution is stable for 24 hours in the dark at room temperature.
4. For Western Blot, use 0.1 mL Working Solution per square centimeter of membrane.
5. Incubate the blot for 5 minutes in the Working Solution.
6. Remove blot from the Working Solution and drain excess liquid.
7. Place the blot in clear plastic wrap and remove bubbles.
8. Expose the blot to X-ray film or use an imaging system.
9. Adjust exposure time to obtain optimal results.

Instructions for Use of the Part A Diluent (CHEMI-DIL)

CHEMI-DIL is a diluent that can be used to dilute Part A to adjust the signal strength to a level consistent with the specific sensitivity and dynamic range requirements of an assay.

Consider testing the following dilutions of the Part A Substrate with the Part A Diluent:

1:2	1 Part Substrate + 1 Part Diluent	50% strength
1:3	1 Part Substrate + 2 Parts Diluent	33% strength
1:4	1 Part Substrate + 3 Parts Diluent	25% strength
1:5	1 Part Substrate + 4 Parts Diluent	20% strength
1:10	1 Part Substrate + 9 Parts Diluent	10% strength

Procedure for use of Diluted Part A:

After dilution, mix equal parts of diluted Part A and undiluted Part B and follow the same procedures for ELISA or Western Blotting.