

Luminol Reagent va-98 (A and B)

Introduction

The Luminol Reagent kit is a non radioactive light-emitting system designed to detect immobilized proteins probed with HRP conjugated either primary or secondary antibody. The method provides a very high sensitivity, yields fast and permanent on X-ray film or on Image Station.

Protocol

Follow the standard procedure to separate your proteins in polyacrylamide gels and transfer them to nitrocellulose membranes.

A. Immunoblotting Procedure

1. Briefly wash the membranes with TBST
2. Block the membranes with 5% Blocking Reagent (va-99) in TBST for 60 min at room temp and gently agitate.
3. Incubate the membranes with primary antibodies diluted with 5% Blocking Reagent (va-99) in Signal Enhancer for 60 min at room temp and gently agitate.
4. Wash the membranes 3 x 5 min with TBST.
5. Incubate the membranes with secondary antibodies diluted with 5% Blocking Reagent (va-99) in Signal Enhancer for 60 min at room temp and gently agitate.
6. Wash the membranes 4 x 5 min with TBST.
7. Briefly wash the membranes with TBS.

B. Chemiluminescent Detection

1. Prepare luminol Working Solution prior to use by mixing in a 1:1 ratio Luminol Reagent A and Luminol Reagent B respectively
2. Transfer the membrane(s) from the last wash to a fresh tray.

3. Pipette the Luminol Working Solution onto the blotted membrane and incubate for 1 minute at room temperature. The membrane should be completely covered with Luminol Working Solution.
4. Remove the membrane(s) from the Luminol Working Solution with forceps, drain excess liquid and seal the membrane(s) with plastic wrap being careful not to have any air bubbles trapped.
5. Develop an image for the Western blotting using either a X-ray film or a digital imaging station.

Storage

Store at 4° C, stable for six months from the date of shipment

Product

Sufficient for 2,000 cm²membrane area

Research Use

For research use only, not for use in diagnostic procedures.

Related Products

Western Blot Signal Enhancer: va-100

Blocking Reagent: va-99

