



HRPL™ Kit

Horseradish Peroxidase Luminescent
Visualization System

- Excellent Sensitivity
- Very Low Background
- Enhanced Stability

INTRODUCTION

Horseradish Peroxidase (HRP) labelling has become the method of choice for many proteomics applications. The sensitivity and convenience of HRP based detection has been greatly enhanced by the use of luminol as a chemiluminescent substrate. HRP catalyzes the reduction of H_2O_2 to H_2O , and then recovers the electrons used for the reduction from luminol, generating an oxidized luminol radical. The luminol radical then reacts with O_2 to create O_2^- . The superoxide anion then reacts with a second luminol radical to produce a luminol endoperoxide, which subsequently decays with emission of light.

By facilitating the series of reactions from HRP + luminol to light emission, National Diagnostics HRPL™ Kit enhances the sensitivity of HRP luminiscent visualization up to 10^4 fold. The HRPL Chemiluminescent Visualization System is designed for the detection and visualization of proteins immobilized on a membrane after electrophoresis or Dot ELISA. The combination of a sensitive, nonradioactive substrate and a highly specific, stable liquid conjugate allows rapid and accurate identification of samples, without the disposal concerns or hazards associated with the use of radioisotopes. The chemiluminescent substrate is provided as a stable two component solution which emits light when combined with HRP-labeled antibody. Positive reaction sites are rapidly and easily detected with high resolution and low background. Results are permanently recorded on X-ray film.

INSTRUCTIONS FOR WESTERN BLOT ASSAY

WORKING SOLUTIONS:

Blocking Solution:

Prepare blocking solution with ProtoBlock Solution (CL-252) or other standard reagent (casein, bovine serum albumin, etc.).

HRPL Reagent:

Mix equal parts of HRPL Solution A and HRPL Solution B. This solution is stable for one hour after mixing. Prepare 1.25 ml of Chemiluminescent Substrate per 10 cm² of membrane.

- 1) Before beginning the immunoassay, use a pen to mark the orientation of the protein samples on the transfer membrane.
- 2) Block transfer membrane by immersing in Blocking Solution (see above) for 30 minutes at room temperature.
- 3) Wash the membrane three times for 5 minutes each at room temperature with 1X Phosphate Buffered Saline (PBS).
- 4) Make appropriate dilution of primary antibody in Blocking Solution and incubate the membrane for 1 hour at room temperature.
- 5) Wash the membrane three to six times for 5 minutes each at room temperature with 1X PBS.
- 6) Make 1:1000 dilution of HRP-conjugated secondary antibody in Blocking Solution and incubate the membrane for 1 hour at room temperature.
- 7) Wash the membrane six times for 5 minutes each at room temperature with 1X PBS.
- 8) Incubate for 1 minute with HRPL Chemiluminescent Reagent (see above).
- 9) Remove membranes from the substrate and drain excess liquid.
- 10) Immediately measure light emission using a camera luminometer or film. If using film, lay membrane flat between sheets of clear plastic and expose X-ray film 1-60 seconds. Be sure that the surface of the membrane is facing the film. DO NOT allow the film to get wet. Be careful that the membrane and film are not moved in relation to one another during exposure.
- 11) Develop film according to manufacturer's instructions.

GUIDELINES FOR USE

The target proteins must be immobilized onto the test membrane prior to application of the system reagents. Membranes suitable for use with this system include polyvinylidene difluoride (PVDF), nitrocellulose, or nylon. For Western Blot, proteins are separated by SDS-Polyacrylamide gel followed by electrophoretic or passive transfer. It may be desirable to stain a test section of the unblocked membrane with a protein stain such as Amido black or Ponceau S to verify attachment of the protein before proceeding with the immunoassay.

- Incubate membrane in the minimal volume of reagents. 10-20 ml of each reagent is required to immerse a 16 X 16 cm membrane in a reaction tray.
- If membrane is cut into strips, immerse each strip into 1-5 ml of solution.
- A platform shaker or rocker should be used during incubation to ensure complete and uniform distribution of assay reagents over the membrane.
- The emission of light is detected using a camera luminometer or any X-ray film suitable for use with radioisotopes.
- Gloves should be worn while handling the membrane and all reagents to avoid contamination with oils or proteins.
- For proper analysis of results, be sure to include positive and negative controls, blanks, and/or protein standards as appropriate.

TROUBLESHOOTING

NO SIGNAL DEVELOPMENT:

- Be sure that all of the steps of the procedure were followed correctly.
- Check that correct orientation of the membrane was maintained during the assay and during film exposure.
- Be sure that no buffers containing sodium azide were used. Azide will inhibit peroxidase activity.
- All buffers should be prepared fresh for best results.

WEAK SIGNAL DEVELOPMENT:

- Optimize primary antibody concentration.
- Increase the amount of protein loaded onto the gel.
- Increase incubation times for primary antibody or incubate at 4°C overnight
- Increase Time of exposure to film.
- Washing in excess of recommended procedures can reduce signal intensity.

EXCESSIVE SIGNAL OR BACKGROUND DEVELOPMENT:

- Optimize antibody dilutions. It is often possible to dilute the conjugate further than 1:1000.
- Decrease the primary antibody or conjugate incubation period.
- Decrease the amount of protein loaded onto the gel.
- Decrease exposure time or delay exposure to the membrane to film.
- Insufficient blocking or washing can cause nonspecific

ORDERING INFORMATION

HRPL™ Western Kit

CL-205	240ml kit (1920 cm ² membrane)
	900ml kit (7200 cm ² membrane)

For Additional Information And Order Placement:

USA:
Toll Free: (800) 526-3867
Georgia: (404) 699-2121
Fax: (404) 699-2077

National Diagnostics, Inc.
305 Patton Drive
Atlanta, GA 30336

EUROPE:
Phone: (44) 01482 646022
(44) 01482 646020
Fax: (44) 01482 646013

National Diagnostics (U.K.)
Unit 4, Fleet Business Park
Itlings Lane, Hessele
East Yorkshire, HU139LX

Int'l © National Diagnostics Inc.

**national
diagnostics**