

LumiSensor™ Plus Chemiluminescent HRP Substrate Kit



Technical Manual No. 0199

Version 03312008

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I. DESCRIPTION

LumiSensor™ Plus Chemiluminescent HRP Substrate Kit renders horseradish peroxidase (HRP) detectable on immunoblots such as Western and Dot blots. The reagents are more sensitive than LumiSensor™ Chemiluminescent HRP Substrate Kit (L00221).

The LumiSensor™ Plus Chemiluminescent HRP Substrate Kit is compatible with and highly recommended for GenScript One-Step Western™ Advanced Kits (L00241, L00242 and L00243) and Western Optimization Kits.

II. KIT COMPONENTS

The kit contains two reagents for HRP signal development, Reagent A (30 ml) and Reagent B (30 ml). It contains enough reagents for 10 mini gel (7.5 x 8 cm) Western or Dot blot detections, or 600 cm² of membrane.

III. KEY FEATURES

- ◆ Easy to perform: The procedure is quick and simple, with only a three-minute incubation period.
- ◆ High sensitivity and low background: The kit is optimized to yield clear results each time.
- ◆ Reproducible results: The kit produces highly reproducible results.

IV. STORAGE

Store the kit at 2 to 8°C. It will remain stable for 12 months.

V. GENERAL PROTOCOL

This kit is designed for the development of membranes from Western and Dot blots after the final wash.

1. Mix one volume of reagent A with one volume of reagent B by vortexing for a few seconds. This forms the working solution. Use 0.1 ml of the working solution per cm² of membrane. The working solution should be warmed up to room temperature before use. The working solution is stable for several hours at room temperature when protected from light.
2. Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching its edge against a tissue. Place the membrane on clean, flat surface, and cover the membrane with the working solution.
3. Incubate for three minutes at room temperature. Place the membrane on a clean tissue. Use a soft clean tissue to remove excess working solution. Wrap the membrane in a clean piece of plastic film.
4. Expose the membrane to a sheet of film for 30 seconds to develop it. Repeat this step with different exposure times for best results.



VI. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

Problem	Probable Cause	Solution
The signal is weak or invisible.	There is too little protein loaded.	Load more protein(s) onto the SDS-PAGE gel.
	Poor transfer efficiency.	Optimize the transfer time and/or the electrical current.
	The incubation time is too short or the reagent is not warm enough.	The working solution should be warmed up to room temperature before use. Increase the incubation time to five minutes to increase the signal intensity.
There is high background or non-specific bands on the blot.	The wash time is too short.	Adding an additional wash step with 1X wash solution always decreases background.
	The signal development time is too long.	Reduce the development time.
	The reagents or equipment have been contaminated.	Use a clean container every time you change the solution before washing. Wear gloves and use clean forceps to handle the membranes.
	There is too much working solution.	Remove excess working solution using a soft clean tissue.

VII. ORDERING INFORMATION

LumiSensor™ Plus Chemiluminescent HRP Substrate Kit: Cat. No. L00225

For Research Use Only.

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