LumiSensor™ Chemiluminescent HRP Substrate Kit

Technical Manual No. 0201 **Version 03282008**

I. DESCRIPTION

LumiSensor™ Chemiluminescent HRP Substrate Kit renders horseradish peroxidase (HRP) detectable on immunoblots such as Western and Dot blots. The reagents are optimized for high sensitivity, high intensity, long duration, and low background.

The LumiSensor™ Chemiluminescent HRP Substrate Kit is compatible with and highly recommended for One-Step Western™ Blot Kits (GenScript, L00204 and L00205).

II. KIT CONTENTS

The kit contains two reagents for HRP signal development, reagent A and reagent B. LumiSensor™ HRP Substrate Kit (GenScript, L00221V60) contains enough reagents for 10 mini gel (7.5 x 8 cm) Western or Dot blot detections, or 600 cm^2 of membrane. LumiSensor™ HRP Substrate Kit (GenScript, L00221V300) contains enough reagents for 3000 cm^2 of membrane, and LumiSensor™ HRP Substrate Kit (GenScript, L00221V500) for 5000 cm^2 of membrane based on 0.1 ml of the working solution per cm^2.

Kit Component	L00221V60 For 600 cm^2	L00221V300 For 3000 cm^2	L00221V500 For 5000 cm^2
Reagent A	30 ml	2 x 75 ml	250 ml
Reagent B	30 ml	2 x 75 ml	250 ml
Protocol	1	1	1

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 - 8°C. It will remain stable for 12 months.

V. 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 cm^2 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 20 seconds to develop it. Repeat this step with different exposure times for best results.

VI. EXAMPLES

Dot blot detection of IL-8 protein.

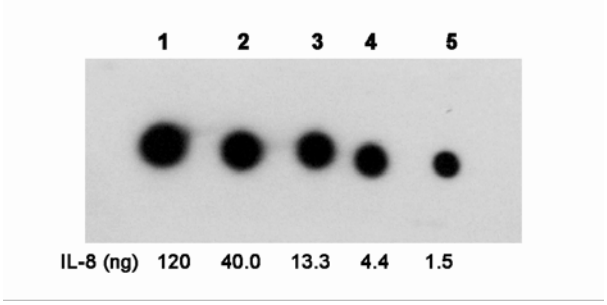

Figure 1. A Dot blot for IL-8 protein using the One-Step Western™ Blot Kit (GenScript, L00205) and Mouse IL-8 Antibody (Endogen, M801). 120 ng, 40.0 ng, 13.3 ng, 7.4 ng, 4.4 ng, and 1.5 ng of IL-8 protein were spotted on the membrane. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate Kit.

Western blot detection of housekeeping protein α-Tubulin using polyclonal antibody.

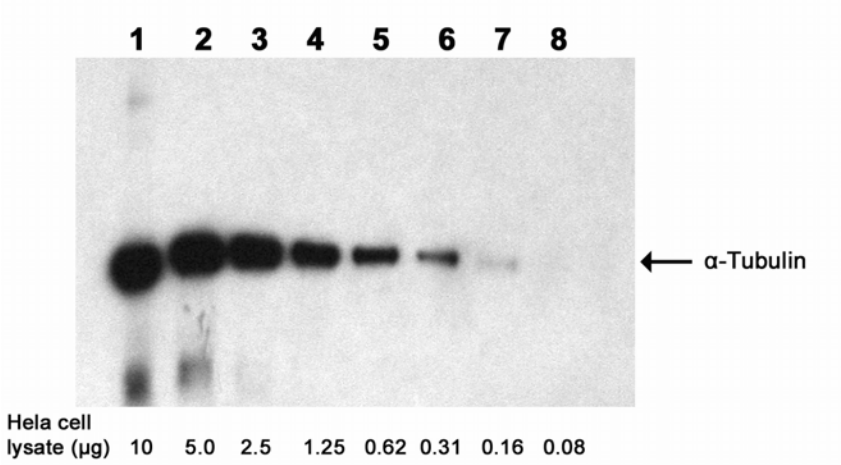

Figure 2. A western blot for α-Tubulin using the One-Step Western™ Blot Kit (GenScript, L00204) and polyclonal α-Tubulin antibody (abcam, ab4074). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg, 0.31 µg, 0.16 µg and 0.08 µg of Hela cell lysate (BD Biosciences, 611449) were loaded into Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, Lane 7, and Lane 8, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate Kit.

A Western blot detection of housekeeping protein GAPDH using monoclonal antibody.

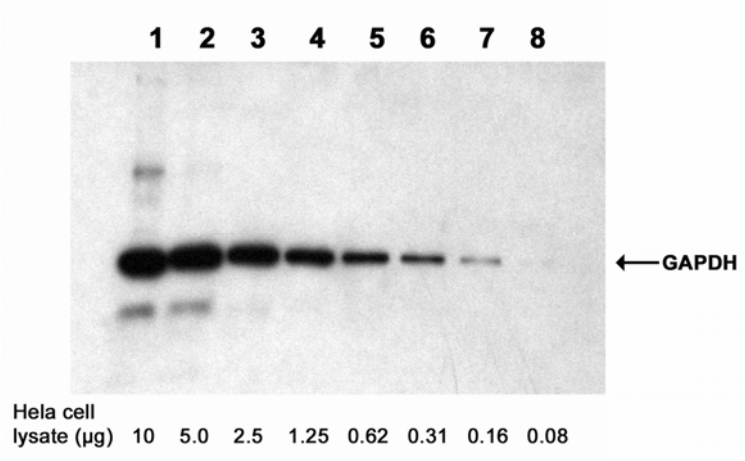

Figure 3. A Western blot for GAPDH using the One-Step Western™ Blot Kit (GenScript, L00205) and Mouse GAPDH Antibody (abcam, ab8245). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg, 0.31 µg, 0.16 µg, and 0.08 µg of Hela cell lysate (BD Biosciences, 611449) were loaded into Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, Lane 7, and Lane 8, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate Kit.

VII. 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

VIII. ORDERING INFORMATION

LumiSensor™ Chemiluminescent HRP Substrate Kit:
L00221V60
L00221V300
L00221V500

For Research Use Only.

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