*pico*LUCENT™ PLUS-HRP

Chemiluminescence Detection System for horseradish peroxidase

INTRODUCTION

*pico*LUCENT™ PLUS-HRP kit is based on our ultra sensitive Luminol substrate that produces chemiluminescence upon reaction with horseradish peroxidase. The chemiluminescence light emission can be recorded by a short exposure to autoradiography films. *pico*LUCENT™ PLUS-HRP kit allows detection of low picogram levels (10^{-12}) of antigens with low noise (signal/background) ratio. The kit reagents are sufficient for 1,000cm^2 of PVDF or nitrocellulose membrane.

KIT COMPONENTS	Cat # 786-09	786-09-R38	786-09-R39	786-09-R40	786-09-R41	786-09-R42	786-09-R48
***pico*LUCENT™ Luminol Solution-A**	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml
***pico*LUCENT™ Peroxide Solution-B**	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml	1 x 50ml
***femto* TBST [10X]**	1 x 250ml	1 x 250ml	1 x 250ml	1 x 250ml	1 x 250ml	1 x 250ml	1 x 250ml
NAP-Blocker [2X]	2 x 250ml	2 x 250ml	2 x 250ml	2 x 250ml	2 x 250ml	2 x 250ml	2 x 250ml
Goat anti Mouse IgG-HRP (786-R38)	N/A	**1 x 2ml**	N/A	N/A	N/A	N/A	N/A
Goat anti Rabbit IgG-HRP (786-R39)	N/A	N/A	**1 x 2ml**	N/A	N/A	N/A	N/A
Goat anti Rat IgG-HRP (786-R40)	N/A	N/A	N/A	**1 x 2ml**	N/A	N/A	N/A
Goat anti Human IgG-HRP (Cat# 786-R41)	N/A	N/A	N/A	N/A	**1 x 2ml**	N/A	N/A
Rabbit anti Goat IgG-HRP (786-R42)	N/A	N/A	N/A	N/A	N/A	**1 x 1.5ml**	N/A
Rabbit anti Human IgG-HRP (786-R48)	N/A	N/A	N/A	N/A	N/A	N/A	**1 x 1.5ml**

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Upon arrival, store the kit components at 4°C, protected from light. When stored and used properly, the kit is stable for 1 year.

ITEMS NOT SUPPLIED WITH THIS KIT:

- Primary antibody
- Secondary antibodies, HRP-conjugates for Cat# 786-09

NOTE: *A wide selections of secondary AP conjugates are available from G-Biosciences (See* ***Related Products****).*

Preparations before Use

A. ***Reconstitute the supplied HRP-conjugates:*** *Depending on your application, re-constitute the supplied HRP conjugate in 2ml or 1.5ml sterile distilled water, stable for several weeks. For extended storage after reconstitution, add an equal volume of glycerol to make final concentration of 50% glycerol and store at -20°C. Avoid repeated freeze/thaw cycles.*

B. ***Preparation of Working Detection solution:*** *Allow the solutions to warm to room temperature before use. For each 8.5cm x 7.5cm membrane, mix 2.0ml of picoLUCENT™ Luminol Solution-A and 2.0ml picoLUCENT™ Peroxide Solution-B*

NOTE: *The mixed solution is stable for 4-6 weeks when stored at 4°C protected from light.*

PROTOCOL

1. Blocking: After the electrophoretic transfer of the protein to an appropriate membrane (e.g. PVDF or Nitrocellulose), block the membrane by immersing in an appropriate blocking buffer. Incubate the blot (membrane) in the blocking buffer for a minimum of 60minutes at room temperature with gentle shaking on an orbital shaker.

> **NOTE:** *Empirical testing to determine the appropriate blocking buffer would prevent non-specific signal with increased sensitivity. We recommend our NAP-Blocker (Cat # 786-190), which produces clear background. If using avidin/biotin system do not use milk as a blocking agent as it contains biotin, which will produce a high background.*

2. Primary Antibody Treatment: Dilute the primary antibody in an appropriate volume of blocking buffer. Incubate the membrane in the diluted primary antibody for 1-2 hours at RT, with gentle shaking. ***NOTE:*** *Determine the optimal dilution of the primary antibody in separate experiments or follow the manufacturer's instructions.*

3. **Washing:** Rinse the membrane twice with TBST or PBST buffer then wash with TBST or PBST buffer 4 times, 5 minutes each and 1x10 minutes at RT, with gentle shaking.

4. **Secondary Antibody Treatment:** Dilute the HRP-conjugated secondary antibody in an appropriate volume of a blocking buffer, at a 1:5,000 to 1:100,000 dilutions. Incubate the membrane in the diluted secondary antibody for 1-2 hours at RT with gentle shaking.

> **NOTE:** *Determine the optimal dilution of the secondary antibody in separate experiments.*

5. Washing: Rinse the membrane twice with TBST or PBST buffer then wash with TBST or PBST buffer 4 times, 5 minutes each and 1x10 minutes at RT, with shaking on an orbital shaker.

6. Chemiluminescence Reaction: Incubate the membrane in the working detection solution for 3-5 minute at room temperature with gentle shaking.

7. Drain the detection reagent and wrap the membrane in saran wrap or clear plastic wrap and expose to an autoradiography film.

> ***NOTE:*** *Do NOT wash or rinse the membrane after addition/removal of the working detection solution.*

8. Place the membrane, (protein side up) in the film cassette. Place a film on top of the membrane and expose the film, initially for 10-20 seconds and then as required.

RE-DEVELOPING THE MEMBRANE (BLOT)

The membrane can be redeveloped within a day or two, provided that the detection reagents are removed from the membrane within 30-60 minutes of the first developing procedure. After each developing procedure, wash the membrane with 50ml 1X *femto*/TBST buffer. Keep the membrane moist and at 4-8^{o}C. Redevelop the membrane according to the protocol above and expose the autoradiography film.

TROUBLESHOOTING

1. No Signal:

a. Protein was not transferred completely from gel to the membrane.
b. Protein is over transferred and passed through the membrane.
c. Primary antibody is not of higher titer or specificity of peroxidase labeled secondary antibody was not appropriate for primary antibody.
d. Use fresh detection reagent and detection buffer.

2. Weak Signal:

a. Antibody concentration was too low or incubation time was too brief.
b. Not enough protein was loaded onto the gel or the primary antibody has low affinity for the target protein.

3. High background, Excessive or Non-Specific Signal:

a. Antibody was not diluted sufficiently or incubation times are excessive (adjust dilution & incubation time).
b. Blocking or washing procedures are inadequate (follow the recommended protocol)
c. The amount of antigenic protein loaded onto the gel is in excess.

STRIPPING AND RE-PROBING MEMBRANE

The developed membrane can be stripped and re-probed with another antibody by using a suitable stripping buffer.
G-Biosciences Western-Re-Probe™ Buffer (5X) is recommended for stripping and re-probing procedures **(See Related Products).**

RELATED PRODUCTS

1 Western-Re-Probe™ [5X] (Cat. # 786-119; 100ml): *Buffer for stripping and re-probing Western blot membranes*

2. BLOT-FastStain™ (Cat# 786-34): *This performs reversible staining of protein on the transfer membranes. It stains only protein and leaves the background absolutely untouched and brilliant white leading to exceptional band visibility. Detects as little as 0.3ng BSA.*

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