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A Geno Technology, Inc. (USA) brand name

*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 >1,500cm^2 of PVDF or Nitrocellulose membrane.

KIT COMPONENTS

Cat. #	HRP tagged Secondary Antibodies	*NAP*-BLOCKER™ [2X]	*femto* TBST [10X]	Luminol Solution-A	Peroxide Solution-B
786-002	-	-	-	50ml	50ml
786-09	-	250ml	250ml		
786-09-R38	Goat anti Mouse IgG, 2ml				
786-09-R39	Goat anti Rabbit IgG, 2ml				
786-09-R40	Goat anti Rat IgG, 2ml				
786-09-R41	Goat anti Human IgG, 2ml				
786-09-R42	Rabbit anti Goat, 1.5ml				
786-09-R48	Rabbit anti Human, 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

- Primary antibody
- Secondary antibodies, HRP-conjugates if Cat. # 786-09 or 786-002 are ordered

PREPARATION BEFORE USE

1. **Preparation of 1X *femto*-TBST:** Dilute the appropriate volume of supplied 10X *femto*-TBST to 1X with DI Water (*e.g. Take 10ml of 10X femto-TBST and add 90ml DI Water to make it 1X).*

2. **Preparation of 1X *NAP*-BLOCKER™:** Use ***aseptic techniques*** for handling *NAP*-BLOCKER™. Allow the supplied 2X *NAP*-BLOCKER™ bottle to come to room temperature and then gently shake to mix. Dilute the appropriate volume of 2X *NAP*-BLOCKER™ 1:1 with 1X *femto*-TBST (*e.g. Take 10ml of 2X NAP*-BLOCKER™ *and add 10ml of 1X femto-TBST*).

3. **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

4. **Reconstitute the supplied HRP-Conjugates:** Depending on your selection of HRP labeled secondary antibody Cat# and application, re-constitute the supplied HRP conjugate in the indicated volume of sterile distilled water.

   NOTE: *The mixed working solution is stable for 4-6 weeks when stored at 4 ℃ protected from light. For extended storage after reconstitution, add an equal volume of glycerol to make final concentration of 50% glycerol and store at -20 ℃. Avoid repeated freeze/thaw cycles.*

PROCEDURE

1. **Blocking:**

After the electrophoretic transfer of the protein to an appropriate membrane (e.g. PVDF or Nitrocellulose), block the membrane by immersing in 10ml 1X *NAP*-BLOCKER™. Incubate the blot (membrane) in the blocking buffer for a minimum of 60 minutes at room temperature with gentle shaking on an orbital shaker.

2. **Primary Antibody Treatment:**

Dilute the primary antibody in an appropriate volume (≤5ml) of 1X *NAP*-BLOCKER™. Incubate the membrane in the diluted primary antibody for 1-2 hours at room temperature, 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 with ~10ml 1X *femto*-TBST then wash three times with ~10ml 1X *femto*-TBST buffer for 10 minutes each at room temperature with gentle shaking.

4. **Secondary Antibody Treatment:**

Dilute the HRP-conjugated secondary antibody in an appropriate volume (≤5ml) of 1X *NAP*-BLOCKER™ at a 1:5,000 to 1:100,000 dilution. Incubate the membrane in the diluted secondary antibody for 1-2 hours at room temperature with gentle shaking.

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

5. **Washing:**

Rinse the membrane with ~10ml 1X *femto*-TBST then wash three times with ~10ml 1X *femto*-TBST buffer for 10 minutes each at room temperature with gentle shaking.

6. **Chemiluminescence Reaction**:

Incubate the membrane in the 4ml 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.*

REDEVELOPING THE MEMBRANE

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 TBS with Tween-20. Keep the membrane moist and at 4-8°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 any other 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 for ordering).*

RELATED PRODUCTS

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

II. ***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. It detects as little as 0.3ng BSA .*

III. ***NAP*-BLOCKER™** *(Cat # 786-190): It's a blocking agent, containing Non-Animal protein, suitable for blocking PVDF and Nitrocellulose membranes.*

IV. ***FASTSilver™*** *(Cat # 786-30): For staining of protein and nucleic acids in acrylamide gels, 100 times more sensitive than the standard Coomassie Blue staining method for protein and 10 fold more sensitive than ethidium bromide for single and double stranded DNA and RNA .*

For additional related products, visit www.GBiosciences.com.

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