G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

# picoLUCENT™ PLUS-AP

## Chemiluminescence Detection System for Alkaline Phosphatase

**(Cat. # 786-09APT, 786-09AP)**

## INTRODUCTION

*pico*LUCENT™ PLUS-AP kit is based on alkaline phosphatase (AP)-chemiluminescence reaction. The chemiluminescence light emission can be recorded by a short exposure to blue-light sensitive autoradiography films. *pico*LUCENT™ PLUS-AP kit allows detection of picogram level of antigens with no background. The kit reagents are sufficient for 25 mini blots of 8.5 x 7.5 cm size each.

## ITEM(S) SUPPLIED

| **Cat. #** | **786-09APT** | **786-09AP** |
|---|---|---|
| **picoLUCENT™ PLUS-AP Detection Reagent** | 10ml | 50ml |
| ***femto*TBST [10X]** | - | 250ml |
| **NAP-Blocker [2X]** | - | 2 x 250ml |

## STORAGE CONDITIONS

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

## ADDITIONAL ITEMS REQUIRED

- Primary antibodies
- Secondary antibodies

## PROTOCOL

For conserving kit reagents, the protocol should be carried out in a container that is just large enough to accommodate the transfer membrane.

***1. Blocking***

After the electrophoresis transfer of the protein to an appropriate membrane, block the membrane by immersing in an appropriate volume of 1X NAP-Blocker diluted in 1X *femto*TBST buffer.

Incubate the membrane in NAP-Blocker solution for at least 60 minutes at room temperature, with gentle shaking on an orbital shaker. Other blocking solutions e.g. BSA, Milk powder (phosphate and toxin free) can also be used.

***2. Primary Antibodies Treatment***

Dilute primary antibodies in an appropriate volume of 1X NAP-Blocker solution. Proper antibody dilution must be empirically determined (See Application Notes).

Incubate the membrane in the diluted primary antibody for 1-2 hours at RT with gentle shaking. (Sealable bag can be used for conserving primary antibody).

***3. Washing***

Rinse the membrane twice in 1X *femto*TBST buffer, then wash with 1X *femto*TBST buffer 3 times, 10 minutes each at RT with gentle shaking.

***4. Secondary Antibodies Treatment***

Dilute AP-conjugated secondary antibodies in an appropriate volume of 1X NAP-Blocker solution (See Application Notes). Incubate the membrane in the secondary antibody for 1-2 hours at RT with shaking.

***5. Washing***

Rinse the membrane twice with 1X *femto*TBST buffer then wash in 1X *femto*TBST buffer (~25ml) 3 times, 10 minutes each at RT with shaking on an orbital shaker.

***6. Developing the Membrane with Detection reagents***

***NOTE****: Allow the Detection Reagent Substrate to warm to room temperature before removing contents.*

Use a sealable bag for conserving reagents. Incubate the membrane (8.5cm x 7.5cm) in 2.0ml *pico*LUCENT™*PLUS*-AP Detection Reagent for 1 minute at room temperature with gentle shaking.

Drain the detection reagent substrate from the reaction bag and wrap the membrane in a saran wrap and expose to an Autoradiography film. **Do not wash the membrane**.

***7. Exposing the Film to the Membrane***

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, depending on band intensity.

## APPLICATION NOTES

Optimal dilution of primary & secondary antibody must be determined by a separate dot blot experiment or following the manufacturer instructions prior to running the *pico*LUCENT™ PLUS-AP protocol ***A. Primary Antibody Dilution***

For primary, *usually* it is 1:500 to 1:2000 for polyclonal antibodies, 1:500-1:1000 for hybridoma supernatant; and 1:1000 to 1:2000 or more for monoclonal antibodies.

***B. Secondary Antibody Dilution***

For AP-conjugated secondary antibodies, *usually* it is 1:1000 to 1:5000 or more.

## REDEVELOPING MEMBRANE

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 5-10°C. Redevelop the membrane according to the protocol above and expose the films.

## STRIPPING AND RE-PROBING MEMBRANE

G-Biosciences's Western ReProbe™ Buffer [5X] is recommended for stripping and re-probing procedures.

After each probing procedure, keep the membrane wet. Prepare 20-25ml solution of 1X Western ReProbe™ in DI water. Incubate the membrane in 1X Western ReProbe™ buffer for 30 minutes at room temperature with shaking. Wash the membrane three times with 1X TBST buffer according to the protocol above. Block the membrane and perform immunodetection according to the above protocol.

## TROUBLESHOOTING

***No Signal***

1. Protein was not transferred completely from gel to the membrane.

2. Protein is over transferred and passed through the membrane.

3. Primary antibody is not of higher titer or specificity of peroxidase labeled secondary antibody was not appropriate for primary antibody.

4. Use fresh detection reagent and detection buffer.

***Weak Signal***

1. Antibody concentration was too low or incubation time was too brief.

2. Not enough protein was loaded onto the gel or the primary antibody has low affinity for the target protein.

***High background, Excessive or Non-Specific Signal***

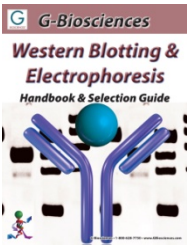
1. Antibody was not diluted sufficiently or incubation times are excessive (adjust dilution & incubation time).

2. Blocking or washing procedures are inadequate (follow the recommended protocol).

3. The amount of antigenic protein loaded onto the gel is in excess.

## RELATED PRODUCTS

Download our Western Blotting Handbook.




http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide

*For other related products, visit our website at www.GBiosciences.com or contact us.*

Last saved: 04/7/2015 SM

*This page is intentionally left blank*


**www.GBiosciences.com**