



# *femto*LUCENT™ PLUS-AP

**Chemiluminescence Detection System  
for Alkaline Phosphatase**

**(Cat. # 786-10AP, 786-10APT)**



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## INTRODUCTION

*femto*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. *femto*LUCENT™ PLUS-AP kit allows detection of femtogram quantity of antigens with no background. The kit reagents are sufficient for 25 mini blots of 8.5 x 7.5 cm size each.

## KIT COMPONENTS

Cat. #	<b>NAP-BLOCKER™</b> [2X]	<i>femto</i> TBST [10X]	<i>femto</i> LUCENT™ PLUS-AP Detection Reagent
786-10APT	-	-	10ml
786-10AP	250ml	250ml	50ml

## 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.

## ADDITIONAL ITEM(S) REQUIRED

- Primary antibody
- Secondary antibodies, AP-conjugates

## 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*).

## 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 ( $\leq 5$ ml) 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  $\sim 10$ ml 1X femto-TBST then wash three times with  $\sim 10$ ml 1X femto-TBST buffer for 10 minutes each at room temperature with gentle shaking.
4. **Secondary Antibody Treatment:** Dilute the AP-conjugated secondary antibody in an appropriate volume ( $\leq 5$ ml) 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  $\sim 10$ ml 1X femto-TBST then wash three times with  $\sim 10$ ml 1X femto-TBST buffer for 10 minutes each at room temperature with gentle shaking.
6. **Chemiluminescence Reaction:** Incubate the membrane in the 2ml femtoLUCENT™ PLUS-AP Detection Reagent 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°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 alkaline phosphatase 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:

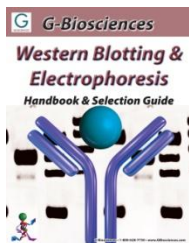
- a. Antibody was not diluted sufficiently or incubation times are excessive (adjust dilution & incubation time).
- b. Blocking or washing procedures are inadequate (follow the recommended protocol).
- c. The amount of antigenic protein loaded onto the gel is in excess.

## 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.

## RELATED PRODUCTS

Download our Western Blotting Handbook:



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

For other related products, visit our web site at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.

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