

Streptavidin AP LumiBlot[™] Kit

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70554-3

Description

The Streptavidin AP LumiBlot Kit is designed for convenient, sensitive detection of immobilized biotinylated proteins and other macromolecules by chemiluminescence. Detection is based on the binding of streptavidin-alkaline phosphatase conjugate (Streptavidin AP) to the biotinylated target molecule, followed by development with CDP-Star[®] substrate. The chemiluminescent signal can be detected by exposure to standard x-ray film, and is also compatible with standard instrumentation having chemiluminescent detection capability. Kit reagents and protocol are optimized for high signal and minimal non-specific background in Western blot applications. Under the conditions described here, less than 1 ng biotinylated protein can be detected in a single band on Western blots.

Components

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| • 50 µl | Streptavidin AP Conjugate |
| • 3 × 200 ml | 20X TBST (3 M NaCl, 200 mM Tris-HCl, 2% TWEEN [®] 20, pH 8.0) |
| • 40 ml | 10% Gelatin |
| • 40 ml | CDP-Star Substrate (includes Nitro-Block [™] II Signal Enhancer) |
| • 25 | gLOCATOR [™] Luminescent Labels |
| • 25 | Development Folders |

Storage

Store gLOCATOR Luminescent Labels and Development Folders at room temperature. Store remaining components at 4°C

Western Blot Detection

This protocol is for the transfer and detection of proteins on nitrocellulose membranes. Note that PVDF or other hydrophobic blotting membranes may require different blocking conditions (e.g., longer blocking incubations, higher concentrations of blocking reagents).

Buffer Preparation

The following describes buffer preparation for a standard 10 × 10 cm blot.

1. Prepare 500 ml 1X TBST by combining 25 ml 10X TBST supplied with the kit with 475 ml deionized water.
2. Prepare 20 ml blocking solution (1% Gelatin in 1X TBST).

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Protocol

The following steps should be performed at room temperature with gentle rocking or agitation during incubations. Place membrane protein-side up. Use containers that have smooth bottom. Lids from large (blue) pipet tip boxes are convenient for use with 7 x 8.5 cm membranes (e.g., Novex). Lids can be washed and reused. Use sufficient volumes of all solutions to cover membranes completely, allowing the membranes to move freely. The recommended incubation times provided are the minimum required. Longer incubation times with Streptavidin AP will not improve signal, but will cause no harm. Likewise, longer wash steps will not cause loss of signal.

1. Run SDS-polyacrylamide gel according to standard protocols. If possible, run positive control sample containing a known biotinylated protein in an adjacent lane. Novagen Perfect Protein™ Western Markers (Cat. No. 69959) provide a convenient set of accurate size standards that can be detected by including S-protein AP Conjugate (Cat. No. 69598) along with the Streptavidin AP Conjugate in Step 4 below.
2. Electrophoretically transfer proteins to nitrocellulose or other suitable membrane. Any standard device can be used according to the manufacturer instructions. The recommended transfer buffer is 96 mM glycine, 12 mM Tris base, 20% methanol, pH 8.3.

Tip: Always use forceps or gloved hands to handle transfer membranes. Handle only at the outer edges to avoid signal anomalies and poor membrane wetting.

3. Remove nitrocellulose from blotting apparatus and incubate, protein side up, in blocking solution at room temperature for 30 min.
4. During blocking incubation, prepare Streptavidin AP dilution. Transfer 50 ml 1X TBST to clean tube. Add 10 µl Streptavidin AP Conjugate for a 1:5,000 dilution. If detection of the Perfect Protein Western Markers is desired, also add 10 µl S-protein AP Conjugate (1:5,000 dilution). Mix gently and maintain at room temperature until the blocking step is complete.
5. Decant blocking solution. Rinse membrane briefly in 30 ml 1X TBST. Decant 1X TBST wash.
6. Add Streptavidin AP dilution. Incubate on rocking platform for 30 min at room temperature.

Tip: Re-use of Streptavidin AP dilutions is not recommended.

7. Following Streptavidin AP incubation, decant conjugate solution. Rinse membrane briefly in 50 ml 1X TBST. Decant. Repeat rinse step with 50 ml 1X TBST. Decant.
8. Add 100 ml 1X TBST. Incubate on rocking platform for 5 min. Discard wash. Repeat twice more with 100 ml 1X TBST, incubating on a rocking platform for 5 min at room temperature each time.

Note: Thoroughly wash membrane to achieve maximum signal:noise ratios.

9. Decant 1X TBST. Allow as much solution as possible to drain. It is helpful to touch the corner of a dry paper towel to the edge of the membrane as it is held on an angle. Place membrane, protein side up, on a sheet of plastic wrap, or in a clean tray.
10. Pipet 1.5 ml CDP-Star® substrate onto surface of membrane. Rock container to evenly wet entire membrane surface. Allow substrate to saturate membrane for 1–2 min.
11. Remove membrane from substrate. Drain excess substrate from membrane by touching membrane to edge of paper towel. Immediately after substrate has been wicked out of membrane (but while it is still moist), place membrane in Development Folder. Remove any bubbles between plastic and membrane. Gently remove any liquid from exterior of the plastic.

Note: Novagen offers products for your convenience. Attach a gLOCATOR™ Luminescent Label to Development Folder. Record any reference information you require on label using felt-tip permanent marker.

12. Place Development Folder in film cassette. To gauge signal intensity, expose to X-ray film for 5 min. Exposure times of up to 30 min are possible without significant background interference.

Tip: The chemiluminescent emission of CDP-Star substrate can be detected with cooled CCD camera-equipped image acquisition systems (e.g., those offered by Nucleovision and Alpha-Innotech) with sensitivities similar to film.