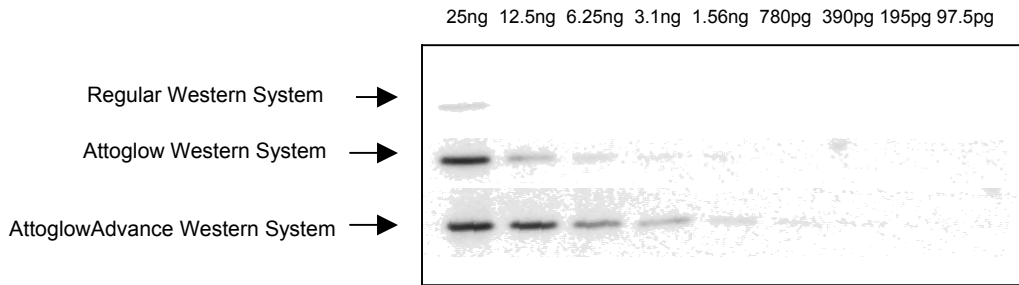


AttoglowAdvance Western Blot Analysis Kit

The most sensitive non-isotope Western blot System

Introduction

Western blot analysis has been widely used in detecting protein expression. Following the magnificent Attoglow Western Blot Analysis Kit, BioChain has developed an even more powerful western detection system: AttoglowAdvance Western Blot Analysis Kit. Combining the advantage of Millennium Enhancer and a super-sensitive system, this kit can be used for detecting extremely low expressed genes.



Comparison of Regular HRP conjugated secondary antibody detection system, Attoglow Western analysis system, and AttoglowAdvance Western analysis system.

Western blots were made by serial diluted GAPDH protein. The three blots were detected by Regular HRP conjugated secondary antibody detection system, Attoglow Western analysis system, and AttoglowAdvance Western analysis system, respectively. The signal was detected by Alphainnotech image system. Expose time is 20 seconds.

Features

- **Super Sensitive** - Detecting low copy genes or increase the sensitivity of poor quality antibodies.
- **Convenient** - Kit contains everything for Western analysis.
- **Versatile** – 100% Enhancement on top of all kinds of chemiluminescent substrates
- **Compatible** - Compatible with PVDF, nitrocellulose, and nylon membranes

Applications

- Detects trace amount of antigen, low expression gene products, useful when poor affinity antibodies are available.
- Western and protein arrays where high sensitivity is needed.
- Combinations with other Western blot enhancers; for an example, in combination with substrate enhancers, enhanced signal magnification is achieved.

Description

This kit provides the following components for whole procedure of Western analysis: 1. Millennium Enhancer; 2. Antibody binding buffer; 3. Attoglow blocking agent; 4. Biotinlated Secondary Antibody; 5. Detection Solution A; 6. Detection Solution B; 7. Luminescence substrate solution A; 8. Luminescence substrate solution B. The components in this kit are prepared with pure chemicals according to our proprietary technology. Four different formats are provided according to the type of secondary antibody that is supplied (Biotin conjugated anti Mouse /anti-Rabbit /anti-Chicken /anti-Goat IgG).

7. Preparation of Detection Solution: for 10 ml Detection Solution, mix 60 µl of detection solution A, 60 µl of detection solution B and 480 µl of PBS. Incubate the mixture at room temperature for 30 minutes. Add the mixture to 10 ml of 1 x TTBS.
8. Incubate blot with Detection Solution prepared by step 7 at RT for 30 min, agitation.
9. Repeat step 4 to remove unbound Detection Solution.
10. Prepare substrate-working solution by mixing equal volume of Substrate Solution A and Solution B right before developing, 2 ml substrate working solution (mixture of 1 ml Buffer A and 1 ml Buffer B) is suggested for a 50 cm² membrane.
11. Incubate blot with substrate working solution for 1-5 min.
12. Place membrane between plastic protection sheets or transparent plastic wrap, and mount inside a film cassette with the protein side facing up.
13. Place a piece of film on top of the membrane, let it expose for an appropriate time, and develop the film. A recommended initial exposure time is 1 min.
14. The blot can be re-developed if necessary.
15. After stripping, the blot can be re-probed.

Re-developing Method

Soak membrane in 1 x TTBS solution at 2-8°C over night. On the second day, (incubate with secondary antibody and apply wash buffer, this step is optional) add substrate and expose on film.

Stripping Method

Use stripping buffer M (See Appendix) if the mild condition is sufficient; and use stripping buffer H (See Appendix) if more stringent stripping conditions are necessary.

1. Soak the membrane in stripping buffer and incubate at 50°C for 30 min with occasional agitation (incubate for a longer time or raise temperature to 70°C if the membrane is not completely stripped).
2. Wash the membrane twice in a large volume of 1 x TTBS for 10 min at room temperature.
3. Repeat the immunoblotting procedure from the blocking step.

Trouble Shooting

1. General Problems

1.1 No signal or weak signal

- Proteins did not transfer properly to membrane
- Not enough protein loaded on the gel
- Target protein degradation occurred due to improper storage of blot
- The concentration of primary or secondary antibody used was too low
- The blocking buffer used was not correct, and antigen was covered
- Substrate had lost activity

1.2 High background

- The concentration of the primary or secondary antibody used was too high
- Too much protein loaded on the gel
- Insufficient blocking
- Insufficient washing
- The level of Tween-20 in blocking buffer was too low
- Membrane problems: e.g., PVDF membrane was not wetted thoroughly or dried in processing
- Transfer buffer been contaminated
- Expose film too long

1.3 Reverse image on film

- Too much antigen
- Too much HRP in the system

2. Kit-specific Problems

2.1 High Background

- A general problem, see above
- The signal was magnified too highly, use our Attoglow Western Blot Analysis kit

Related Products

Attoglow Western Analysis System, Protein array, Total protein, Compartment Proteins

Appendix

Preparation of solutions not supplied with kit:

Solution	Preparation	Stability/temperature	Notes
1 x TTBS	Add 6.05 g Tris base (50 mM), 8.76 g sodium chloride (150 mM) to 800 ml distilled water, adjust pH to 7.5 with HCl; adjusted to 1 liter with distilled water. Add Tween-20 to 0.1% (v/v)	3 months at RT	Do not use sodium azide as an antimicrobial agent as it inhibit HRP
Blocking Solution	Weigh 5 g of non-fat dry milk and dissolve it in 100 ml 1 x TTBS solution	Freshly made suggested	Can be Stored at 2-8°C O/N
Stripping Buffer M	100 mM Glycine, pH 2.7	1 month at RT	Mild stripping buffer
Stripping Buffer H	62.5 mM Tris-HCl, pH 6.7 with 2% SDS and 100 mM 2-Mercaptoethanol	1 month at RT	Harsh stripping buffer