BioChain®
www.biochain.com
Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

User's Manual and Instructions

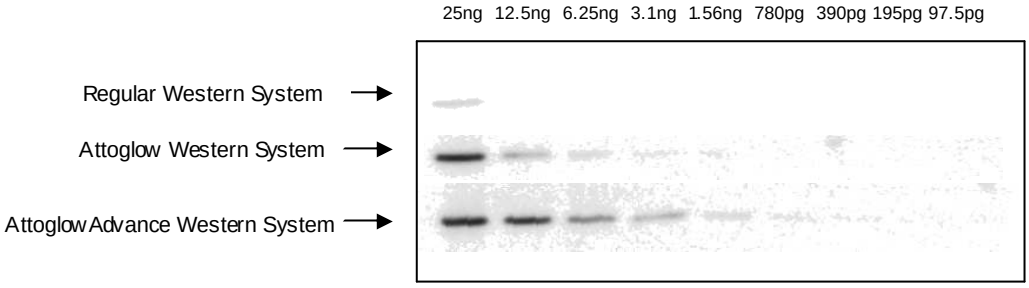
AttoglowAdvance Western Blot Analysis Kit

The most sensitive non-isotope Western blot System

Catalog Number: K3173120-I, K3173120-II, K3173120-III, K3173120-IV

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).

Quality Control

Duplicated Western blots containing 25 ng, 12.5 ng, 6.25 ng, 3.1 ng, 1.56 ng, 780 pg, 390 pg, 195 pg, and 97.5 pg are detected with anti-GAPDH antibody. Expose the membrane Alphainnotech image system for 20 seconds; the signal generated by AttoglowAdvance Western Analysis kit should be much stronger than that of Attoglow Western Analysis kit.

Note: Both kits are very sensitive at such high level amount of protein, one second exposure to x-ray file may saturate the signals and the differentiated signals cannot be visualized.

Components (sufficient for 1,200 cm² membranes)

Catalog Number: K3173120 (sufficient for 1,200 cm² membranes)

Item	Amount	Part No.
1. ***Millennium Enhancer***	50 ml	K3173120-1
2. Antibody Binding Buffer (20 x)	60 ml	K3173120-2
3. Attoglow Blocking Agent	20 g	K3173120-3
4. Biotinlated Secondary Antibody (anti-Mouse or anti-Rabbit or anti-Chicken or anti-Goat IgG)	200 µl	K3173120-4
5. Luminescence substrate solution A	25 ml	K3173120-5
6. Luminescence substrate solution B	25 ml	K3173120-6
7. Detection Solution A	1.2 ml	K3173120-7
8. Detection Solution B	1.2 ml	K3173120-8

Items needed, but not supplied:

1. Primary antibody.
2. Wash buffer (1 x TTBS).
3. 1 x PBS, pH 7.4
4. Membrane stripping and re-probing buffer.

Storage and Stability

Solutions 1, 2, and 3 can be stored at room temperature. Solutions 4, 5, 6, 7 and 8 should be stored at 2-8°C. The kit is stable for one year when handled properly.

Protocol

1. Remove blot from the transfer apparatus and soak in transfer buffer. Under the hood, pour the Millennium Enhancer solution into a new container. For a 50 cm^2 blotting membrane, use 10 ml Millennium Enhancer, and the solution can be re-used 4 times without losing its enhancing effect. Pick the membrane from the transfer buffer and drain the remaining buffer on the membrane as much as possible. For best results, do not let the membrane dry completely. Soak the membrane in the Millennium Enhancer solution, agitate for 2 min, then remove the membrane and submerge it in 1 x TTBS solution.
2. Block the membrane with 5% (v/w) Attoglow blocking agent in 1 x antibody binding solution for 30 min at room temperature with agitation.
3. Prepare antibody binding working buffer by diluting the supplied 20x antibody binding stocking buffer 20 times with ddH_2O. Make an appropriate dilution of primary antibody in antibody binding working buffer; 10 ml of primary antibody solution volume is suggested for a 50-cm^2 membrane. Remove the blocking reagent and add the primary antibody solution. Incubate the blot with agitation for one hour at room temperature or overnight at 2-8°C.
4. Wash the membrane in 1 x TTBS for 5 min, repeat 3-4 times.

5. Add 1 µl of Biotinlated secondary antibody to 1ml of antibody binding working buffer to become 1:1000 dilution. Incubate blot in the diluted solution for 30 minutes to 1 hour at RT with agitation. 10 ml of Biotinlated secondary antibody solution is suggested for membrane size of 50 cm^2. If high background is generated, use higher diluted Biotinlated secondary antibody solution.
6. Repeat step 4 to remove unbound Biotinlated secondary antibody.
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 cm^2 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

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