One-Step Advanced Western™ Kit

Technical Manual No. 0223 **Version 04072008**

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I. DESCRIPTION

One-Step Advanced Western™ Kit yields a journal-quality Western or Dot blot in about one hour. Using GenScript's breakthrough immunodetection technology (patent pending), the kit replaces the classical three-step Western process, which can take nearly five hours. Transfer the proteins from gel to membrane and incubate it in the pretreat solution for five minutes. Then incubate in WB solution with primary antibody for 40 minutes, and lastly, wash three times for ten minutes each. The membrane can then be developed with the HRP substrate included in the kit. The kit contains all the necessary reagents, buffers, nitrocellulose membrane and HRP substrate for performing a Western blot. The One-Step Advanced Western™ procedure is contrasted with a classical three-step Western at right.

The One-Step Advanced Western™ Kit is designed to produce high signal with low background for quick and clear Western analysis of proteins.

The kit contains WestClear™ Nitrocellulose Membrane (0.2 µm) and LumiSensor™ Plus Chemiluminescent HRP substrate optimized for best results. WestClear™ Nitrocellulose Membrane and LumiSensor™ Plus Chemiluminescent HRP Substrate Kits are also available separately.

This kit is not recommended for use with antibodies against phosphoprotein.

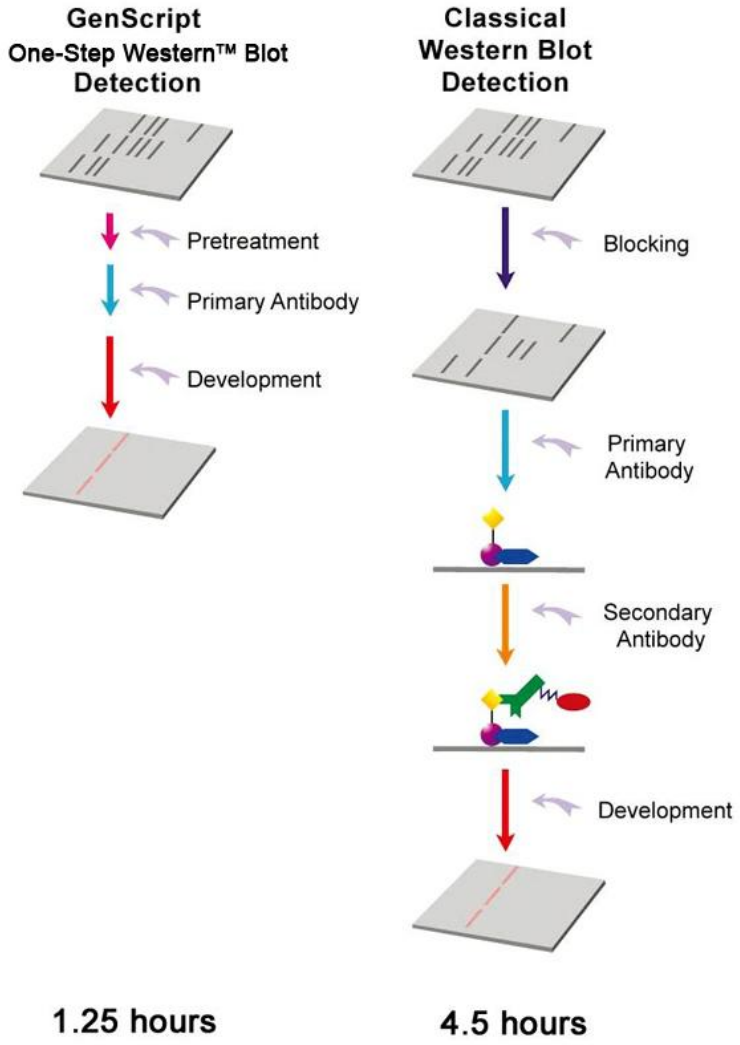


Figure 1. Overview of Western Procedures

II. KIT CONTENTS

Three different kinds of One-Step Advanced Western™ Kits are available, for use with rabbit (GenScript, L00241), mouse (GenScript, L00242), and goat (GenScript, L00243) primary antibodies, respectively. Each kit contains enough reagents for ten minigel (7.5 x 8 cm) Western blots.

Kit Components	10 Assays L00241 (Rabbit)	10 Assays L00242 (Mouse)	10 Assays L00243 (Goat)
Pretreat A solution	100 ml	100 ml	100 ml
Pretreat B solution	100 ml	100 ml	100 ml
WB-1 solution	1 ml	1 ml	1 ml
WB-2 solution	100 ml	100 ml	100 ml
10X Wash solution	125 ml	125 ml	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 Sheets	10 Sheets	10 Sheets
LumiSensor™ Plus Chemiluminescent HRP Substrate	2 x 30 ml	2 x 30 ml	2 x 30 ml
Dot Blot Box	1	1	1
Protocol	1	1	1

III. RELATED PRODUCTS

- WestClear™ Nitrocellulose Membrane L00224A60
- LumiSensor™ Chemiluminescent HRP Substrate Kit L00221V60
- LumiSensor™ Plus Chemiluminescent HRP Substrate Kit L00225
- 10X Wash Solution MB01011
- Pretreat Solution (A + B) M01013
- GenScript Dot Blot Box M00108

IV. KEY FEATURES

- Easy to perform: This kit has fewer and simpler steps than other Western kits, leaving fewer chances for human error.
- Low background: The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ Plus Chemiluminescent HRP Substrate Kit, optimized for low background.
- High sensitivity: The kit's sensitivity is higher than that of the classical 4.5-hour procedure, depending on the quality and amount of antibodies used.
- Reproducible results: The kit produces highly reproducible results.
- No secondary antibody is needed.
- The One-Step Western™ needs far less optimization than the classical three-step method.

V. STORAGE

Store WestClear™ Nitrocellulose Membrane at room temperature. Store the rest of the kit at 4°C. It will remain stable for six months. **Do not freeze the kit or any of its components**.

VI. ONE-STEP WESTERN™ PROTOCOL

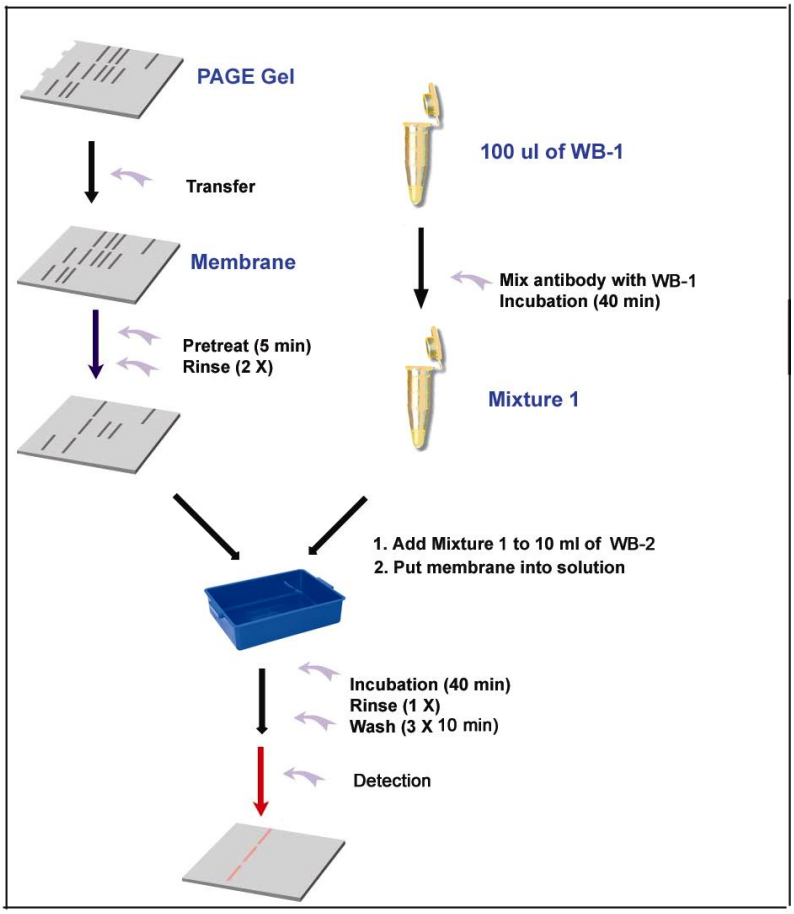
This procedure is optimized for a sheet of 7.5 x 8 cm membrane, but reagent volumes can be scaled according to the size of the membrane used.

Reagents not provided:

Purified primary antibodies. Affinity-purified antibodies are recommended. Further optimization may be needed if the serum containing the antibody is to be used.

Before use, prepare the following:

Dilute 12.5 ml of 10X wash solution with 112.5 ml of distilled or filtered water to make 125 ml of 1X wash solution. Use 15 ml of 1X wash solution for each rinse and 20 ml of 1X wash solution for each wash. If any precipitate forms in 10X wash solution during storage, incubate the bottle in warm or hot water bath (up to 50°C) with occasional mixing until all the precipitate disappear. Dilute the buffer with ddH_2O to 1X and store it at 4°C.



Antibody Concentration Titration Test

Due to the varying affinity and specificity of antibodies and the differing sensitivity of imaging films (the film of one brand can be several folds more sensitive than that of other brand) that are used for signal imaging, antibody concentration titration is highly recommended for best results.

Follow the procedure as described below using GenScript Dot Blot Box (M00108) to perform the antibody concentration titration. The Dot Blot Box, which can process up to five 7.5 x 1.5 cm strips of membrane, can both conserve reagents and improve precision.

1. Load the same amount of protein sample into three wells of a 10-well minigel.
2. After transferring proteins to the membrane, cut the membrane to three small strips. For each slot of the Dot Blot Box, use 2 ml of fresh pretreat solution mixture (pretreat A plus pretreat B) for pretreatment of the strips.
3. Prepare these three different mixture 1 solutions in three 1.5 ml of centrifuge tubes and use them for primary antibody titration test: a). Add 1 µg of primary antibody to 10 µl of WB-1 Solution and mix well. b). Add 0.5 µg of primary antibody to 5 µl of WB-1 Solution and mix well. c). Add 0.2 µg of primary antibody to 2 µl WB-1 Solution and mix well. Incubate Mixture 1 at RT (room temperature) for at least 40 minutes, and then add 1 ml of WB-2 to each tube. These solutions will be used for final incubation of pre-treated membrane strips.
4. Process the membrane as described in the following procedure and select the best antibody and WB-1 amount for future Western blot analyses.

Western blot procedure:
Do not wash the membrane after transferring the proteins from the gel. Proceed directly to the steps below.

1. Prepare Mixture 1

Before or during protein transfer, prepare mixture 1 by mixing 2 to 10 µg of primary antibody with 20 to 100 µl of WB-1 (as determined by titration test) in a microcentrifuge tube. Vortex mixture 1 for a few seconds and spin down briefly to collect the solution in the bottom of the tube. Incubate mixture 1 at RT (room temperature) for at least 40 minutes. (Longer incubation is preferred. For overnight incubation, store mixture 1 at 4°C.)

2. Pre-Treat Membrane

Just before the protein transfer from gel to membrane is complete, mix 10 ml of pretreat A solution with 10 ml of pretreat B solution in a plastic container to make the pretreat solution. Incubate the membrane in the pretreat solution mixture on a shaker for five minutes at RT. After incubation, rinse the membrane twice with 15 ml of 1X wash solution.

3. Final Incubation of Pre-Treated Membrane

a. Add mixture 1 to 10 ml of WB-2 in a plastic container and mix well. Incubate the membrane in this solution (WB-2 containing mixture 1) on a shaker at RT for 40 minutes. This mixture (WB-2 containing mixture 1) can be recovered and reused up to three times if stored at 4°C. However, this may cause variations to arise due to changes in antibody concentration and carryover contamination.
b. Rinse the membrane once with 15 ml of 1X wash solution. Wash the membrane on a shaker three times for ten minutes each with 20 ml of 1X wash solution. **Use a clean container for each wash step to avoid carryover contamination and to reduce background.**

4. Signal Development

a. Mix 3 ml of LumiSensor™ Plus Reagent A with 3 ml of LumiSensor™ Plus Reagent B by vortexing for a few seconds to make the working solution. Use 0.1 ml of the working solution per cm^2 of membrane. The working solution is stable for several hours at room temperature when protected from light.
b. Drain the excess wash solution from the membrane by holding the membrane vertically with forceps and touching the edge against a tissue. Place the membrane on clean, flat surface, and cover the membrane with working solution.
c. Incubate for three minutes at room temperature. Place the membrane on a soft, clean tissue. Use another tissue to remove excess working solution. Wrap the membrane in a clean piece of plastic film.
d. Expose to a sheet of film for 30 seconds and then develop. Repeat with different exposure times to find the best results.

VII. EXAMPLES

Comparison of One-Step Western™ blot with classical Western blot using rabbit polyclonal antibody:

One-Step Advanced Western™ blot was compared to a classical Western blot for the detection of a His-tagged protein from *E. coli* cell lysate. Two similar blots were processed with different procedures using Rabbit Anti-His-tag Polyclonal Antibody (GenScript, A00174): Classical Western blot detection (4.5 hours, left panel, figure 2) and One-Step Advanced Western™ blot (1.25 hours, right panel, figure 2).

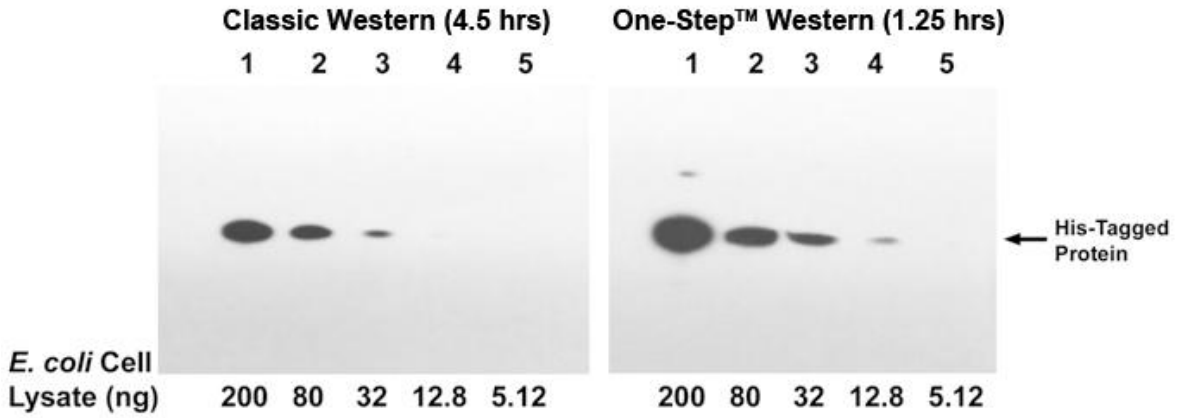


Figure 2. Western blots for the detection of a His-tagged protein from *E. coli* cell lysate by both classical Western and One-Step Western™ using the One-Step Advanced Western™ Kit L00241. 200, 80, 32, 12.8, and 5.12 ng of total protein was loaded into lanes 1, 2, 3, 4, and 5, respectively. The classical Western blot was developed with LumiSensor™ Chemiluminescent HRP Substrate (GenScript, L00221V60) and the One-Step Western™ blot was developed with the LumiSensor™ Plus Chemiluminescent HRP Substrate included in the kit L00241.

Compared with classical Western blot that takes more than 4.5 hours, One-Step Advanced Western™ Kit, which takes only 1.25 hours for the detection of protein, produces higher signal with very low background.

VIII. TROUBLESHOOTING

Problem	Probable Cause	Solution
The signal is weak or invisible.	Too little protein is loaded.	Load more protein(s) onto the SDS-PAGE gel.
	There is poor transfer efficiency.	Optimize the transfer time and/or the electrical current. Make sure that there are no air bubbles between the membrane and the gel.
	The primary antibody has a low affinity for the antigen.	Increase the incubation time of the membrane in WB-2 containing mixture 1 from 40 to 50 or 60 minutes.
High background	The primary antibody shows non-specific binding or cross-reactivity.	Change antibodies. Use a highly specific primary antibody. Affinity-purified primary antibodies are preferred.
	There is too much primary antibody.	Perform antibody concentration titration test to find the optimal conditions.
	The wash time is too short.	Adding additional washings after primary antibody (in WB) binding can further decrease background.
	The signal development time is too long.	Reduce the exposure time. If both the signal and background are high, wait for a few minutes before exposing the film.
	The equipment or reagents have become contaminated.	Use a clean container for each rinse and wash step. Wear gloves and use clean forceps to handle membranes.

IX. ORDERING INFORMATION

One-Step Advanced Western™ Kit: Cat. No. L00241 for rabbit primary antibody.
Cat. No. L00242 for mouse primary antibody.
Cat. No. L00243 for goat primary antibody.

Patent Pending.

For Research Use Only.

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