



I	Description	1
II	Key Features	1
III	Kit Contents	2
IV	Storage	2
V	Related Products.....	2
VI	One-Step Western™ Protocol	3
VII	Examples	4
VIII	Troubleshooting.....	5

I. DESCRIPTION

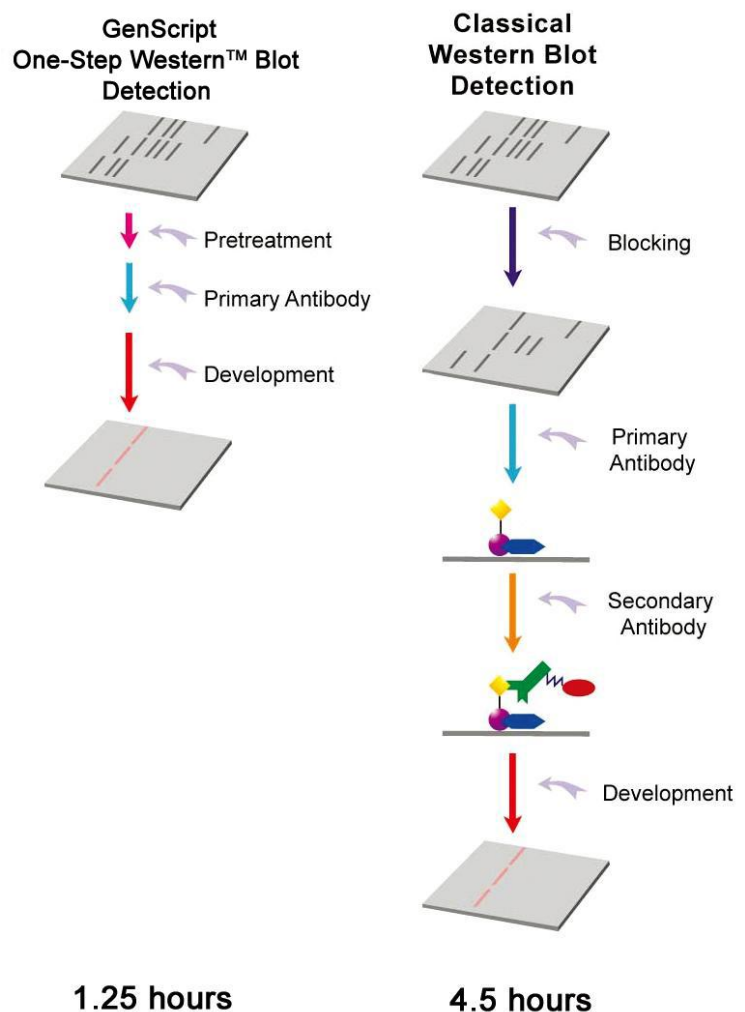
GenScript One-Step Western™ Fluorescent Kit yields journal-quality Western or Dot blot results in about one hour. Using GenScript breakthrough immunodetection technology (patent pending), the kit replaces the classical three-step Western process, which can take nearly five hours. Transfer the proteins from gel to membrane and incubate it in the pretreat solution for five minutes. Then incubate in WB solution with primary antibody and fluorescent dye labeled secondary antibody for 40 minutes. Then wash three times for ten minutes each. The membrane can then be scanned on a LI-COR Odyssey Infrared Imaging Systems. The One-Step Western™ procedure is contrasted with a classical three-step Western at right.

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

This kit can be used for any primary antibodies from any species. An appropriate fluorescent dye labeled secondary antibody is also needed.

II. KEY FEATURES

- ◆ Easy to perform: This kit has fewer and simpler steps.
- ◆ Reproducible results: The kit produces highly reproducible results.
- ◆ No dark room, developer or X-Ray film is needed.



Overview of Western Procedures

III. KIT CONTENTS

Each kit contains enough reagents for ten minigel (7.5 X 8 cm) Western blots.

In some rare cases, the primary antibody (including some antibodies against phosphoproteins) may not be compatible with Pretreat Solution A, resulting in very high background. For these cases, GenScript provides an alternate pretreat solution A (Pretreat A-b) to solve this problem. Pretreat A-b (100 ml, GenScript, M01057) is available separately.

Kit Components	Quantity
Pretreat Solution A	100 ml
Pretreat Solution B	100 ml
WB-1 Solution	2 ml
WB-2 Solution	100 ml
10X Wash Solution	125 ml
Protocol	1

Either Nitrocellulose or PVDF membrane (not provided) can be used with the kit.

IV. STORAGE

Store the kit at 4°C. It will remain stable for six months.

V. RELATED PRODUCTS

- WestClear™ Nitrocellulose Membrane (0.2 μm) L00224A60
- 10X Wash Solution MB01011
- Pretreat A-b M01057
- GenScript Dot Blot Box M00108
- Western Blot Box (Black) M00103
- Protein marker for Fluorescent Western M00124
- 5X Sample Buffer for SDS-PAGE MB01015

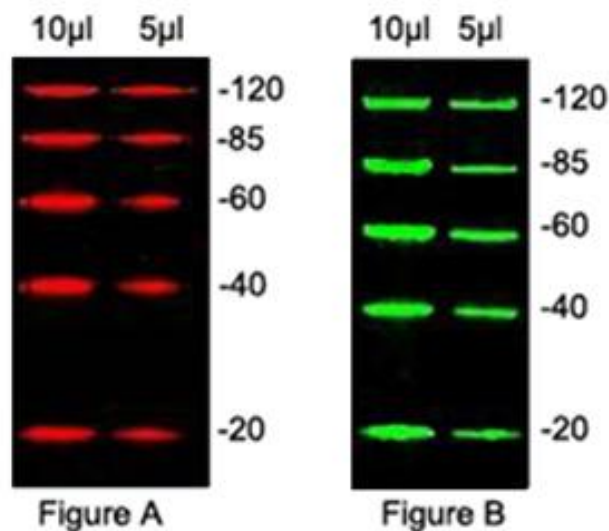
Protein marker for Fluorescent Western

GenScript also provides protein marker for Fluorescent Western (M00124), the performance of the marker is demonstrated at right.

10 μl and 5 μl of reconstituted protein marker for Fluorescent Western blot were loaded in 12% Express SDS-PAGE Gel (GenScript, MG012W10).

Fig. A shows the results when IRDye 680-conjugated Goat Anti-Rabbit IgG (LI-COR) is used in the Western blot.

Fig. B shows the results when IRDye 800CW-conjugated Goat Anti-Mouse IgG (LI-COR) is used in the Western blot. Images were acquired using Odyssey Infrared Imaging system (LI-COR).



VI. ONE-STEP FLUORESCENT WESTERN™ PROTOCOL

This procedure is optimized for a sheet of 7.5 X 8.0 cm membrane, but reagent volumes can be scaled according to the size of the membrane used.

Reagents not provided:

1. Purified primary antibodies: Affinity-purified antibodies are recommended.
2. Fluorescent dye labeled secondary antibodies. Several vendors provide this kind of antibodies. LI-COR and Rockland provide IRDye® 680/800 labeled secondary antibodies. Pierce provides DyLight 680/800 labeled secondary antibodies. Invitrogen provides Alexa Fluor® 680 labeled secondary antibodies.

Before use, prepare the following:

1X wash solution: 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. If any precipitate forms in the 10X wash solution during storage, incubate the bottle in a warm or hot water bath (up to 50°C) with occasional mixing until all the precipitate disappears. Dilute the buffer with ddH₂O to 1X and store it at 4°C. Use 15 ml of 1X wash solution for each rinse and 20 ml of 1X wash solution for each wash.

Western blot procedure:

1. Prepare Mixture 1

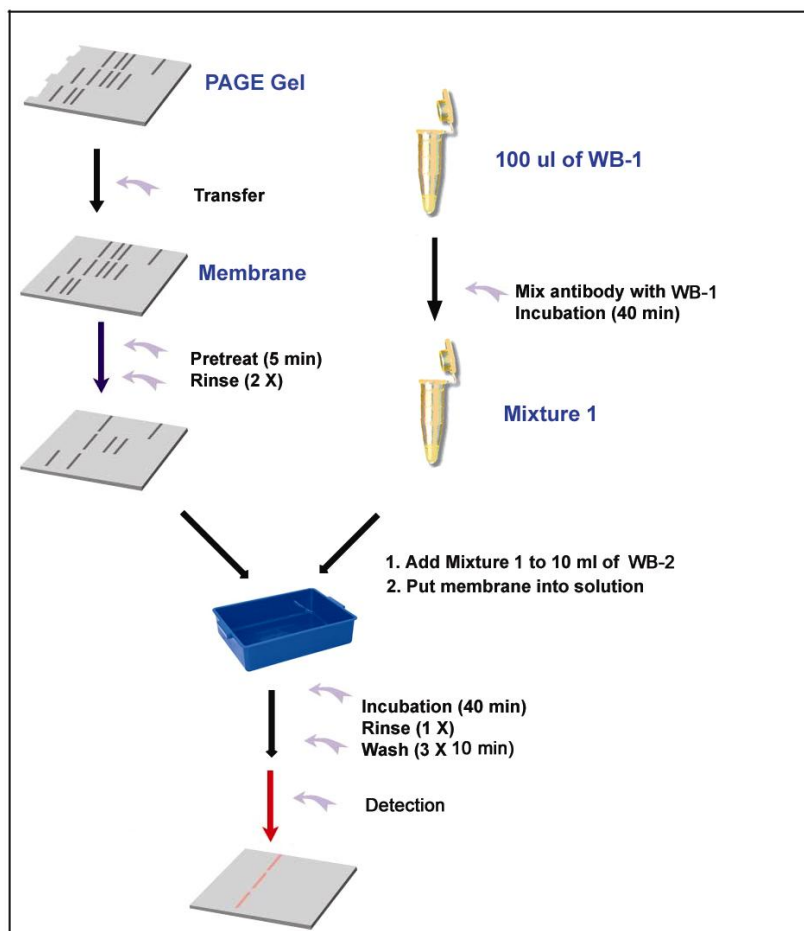
Before or during protein transfer, prepare Mixture 1 by mixing primary antibody and Fluorescent dye labeled secondary antibody in WB-1. Add 2 – 10 µg of primary antibody* to 100 µl of WB-1 in a microcentrifuge tube, then add 1–5 µg of Fluorescent dye labeled secondary antibody (the amount of secondary antibody is 50% of the primary antibody used) to the same tube. Vortex Mixture 1 gently for a few seconds and centrifuge briefly. Incubate Mixture 1 in the dark at room temperature (RT) for at least 40 minutes.

* Refer to manufacturer's recommendations when using appropriate amounts of antibody.

2. Pre-Treat Membrane

Just before the protein transfer from gel to membrane is complete, mix 10 ml of Pretreat Solution A with 10 ml of Pretreat Solution B in a plastic container (Western blot box, GenScript, M00100) to make the pretreat solution. Always prepare and use fresh mixture. Place the membrane directly in the pretreat solution mixture and incubate 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 Western blot box (GenScript Western Blot Box, Black, M00103) and mix well. Incubate the membrane in this solution (WB-2 containing mixture 1) on a shaker at RT for 40 minutes. **Protect box (or bag) from light during incubation.** This mixture (WB-2 containing mixture 1) may 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. **Protect box (or bag) from light during wash. Use a clean container for each wash to reduce background.**

4. Imaging or Scanning

After final wash, transfer the membrane to a container containing 20 ml of distilled or filtered water. Rinse the membrane for 1 minute and then scan the membrane on a LI-COR Odyssey Infrared Imaging Systems following the Odyssey Operation Manual.

VII. EXAMPLES

Fluorescent Western blot detection of GST using rabbit polyclonal antibody

GST protein was detected using One-Step Western™ Fluorescent Kit (L00397). The Mixture 1 was prepared by mixing 10 µg of Rabbit Anti-GST-tag Polyclonal Antibody (GenScript, A00097) with 5.0 µg of IR®800 Goat anti-Rabbit (LI-COR) in 100 µl of WB-1.

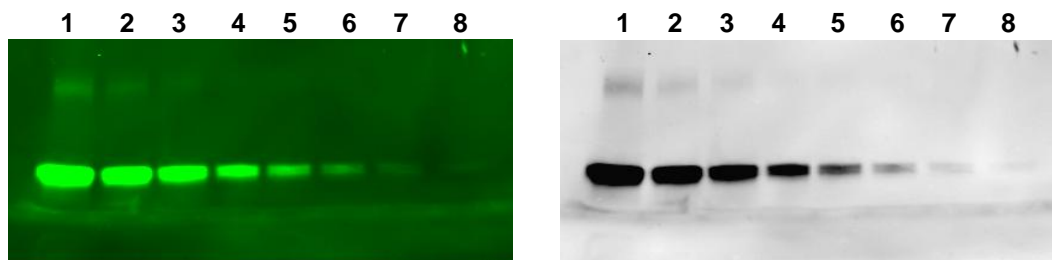


Figure 1. Fluorescent Western blots for the detection of GST protein using the One-Step Western™ Fluorescent Kit (L00397). 50.0, 25.0, 12.5, 6.25, 3.12, 1.56, 0.78 and 0.39 ng of GST protein were loaded into Lanes 1, 2, 3, 4, 5, 6, 7 and 8 respectively.

Fluorescent Western blot detection of housekeeping protein GAPDH using goat polyclonal antibody

One-Step Western™ Fluorescent Kit (L00397) was used to detect GAPDH protein in Hela cell lysate. The Mixture 1 was prepared by mixing 6 µg of Goat Anti-GAPDH Polyclonal Antibody (GenScript, A00191) with 3 µg of IR®800 Donkey anti Goat (LI-COR) in 100 µl of WB-1.

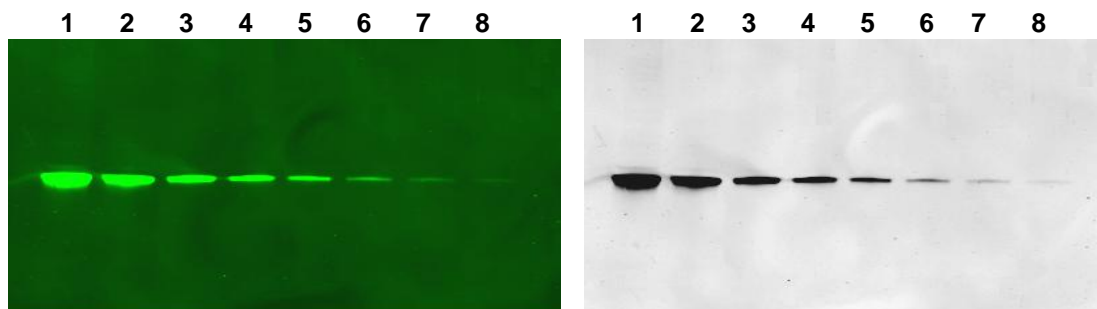


Figure 2. Fluorescent Western blots for the detection of GAPDH using the One-Step Western™ Fluorescent Kit (L00397). 5.0, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08 and 0.04 µg of *HeLa* cell lysate were loaded into Lanes 1, 2, 3, 4, 5, 6, 7 and 8 respectively.

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. Increasing antibody concentration can also improve signal.
	The primary antibody has a low affinity for the antigen.	Reducing wash time can increase the signal for low-affinity antibody. Instead of wash for 3 x 10 min, wash for 3 x 5 min to increase signal.
There is high background.	Too much primary antibody is used.	Reduce the amount of primary antibody, and reduce WB-1 accordingly.
	The primary antibody has non-specific binding or cross-reactivity with the blocking reagent.	Use an alternate Pretreat A-b (M01057).
	The wash time is too short.	Adding additional washing steps can further decrease background.
	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.

Patent Pending.

For Research Use Only.

GenScript Corporation
 860 Centennial Ave., Piscataway, NJ 08854
 Tel: 732-885-9188, 732-885-9688
 Fax: 732-210-0262, 732-885-5878
 Email: info@genscript.com
 Web: www.genscript.com

Limited Use Label license: This product may be the subject of one or more patents filed by GenScript Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for any Commercial Purposes. For commercial use, please contact GenScript at info@genscript.com.