

ONE-HOUR Western™ Kit

Technical Manual No. 0352

Update date 06192009

I	Description	1
II	ONE-HOUR Western Kit Selection Guide.....	2
III	Kit Contents	3
IV	Related Products	3
V	Key Features.....	4
VI	Storage.....	4
VII	ONE-HOUR Western™ Protocol	4
VIII	Examples	6
IX	Troubleshooting.....	7

I. DESCRIPTION

GenScript's ONE-HOUR Western™ Kit yields journal-quality Western or Dot blot results 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-2 solution with the mixture of primary antibody and WB-1 solution for 40 minutes. Then wash three times for five or ten minutes each. The membrane can then be developed with the HRP substrate. The ONE-HOUR Western™ procedure is contrasted with a classical three-step Western at right.

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

Except for basic kits, all other kits contain all the necessary reagents, buffers and materials such as membrane and X-ray films for performing a Western blot, and no additional secondary antibody is needed. The ONE-HOUR Western™ Kit saves precious research time. For added cost-efficiency, we recommend our complete kits, which contain all the reagents, membrane, HRP substrate, and X-ray film necessary for a perfect blot. However, the kit components are also sold separately.

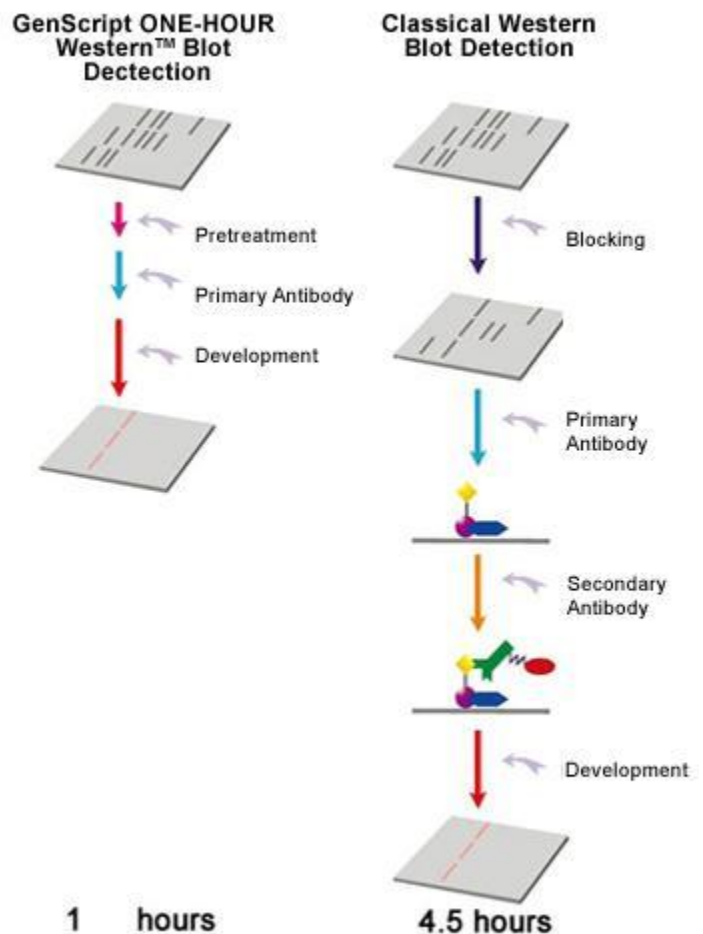
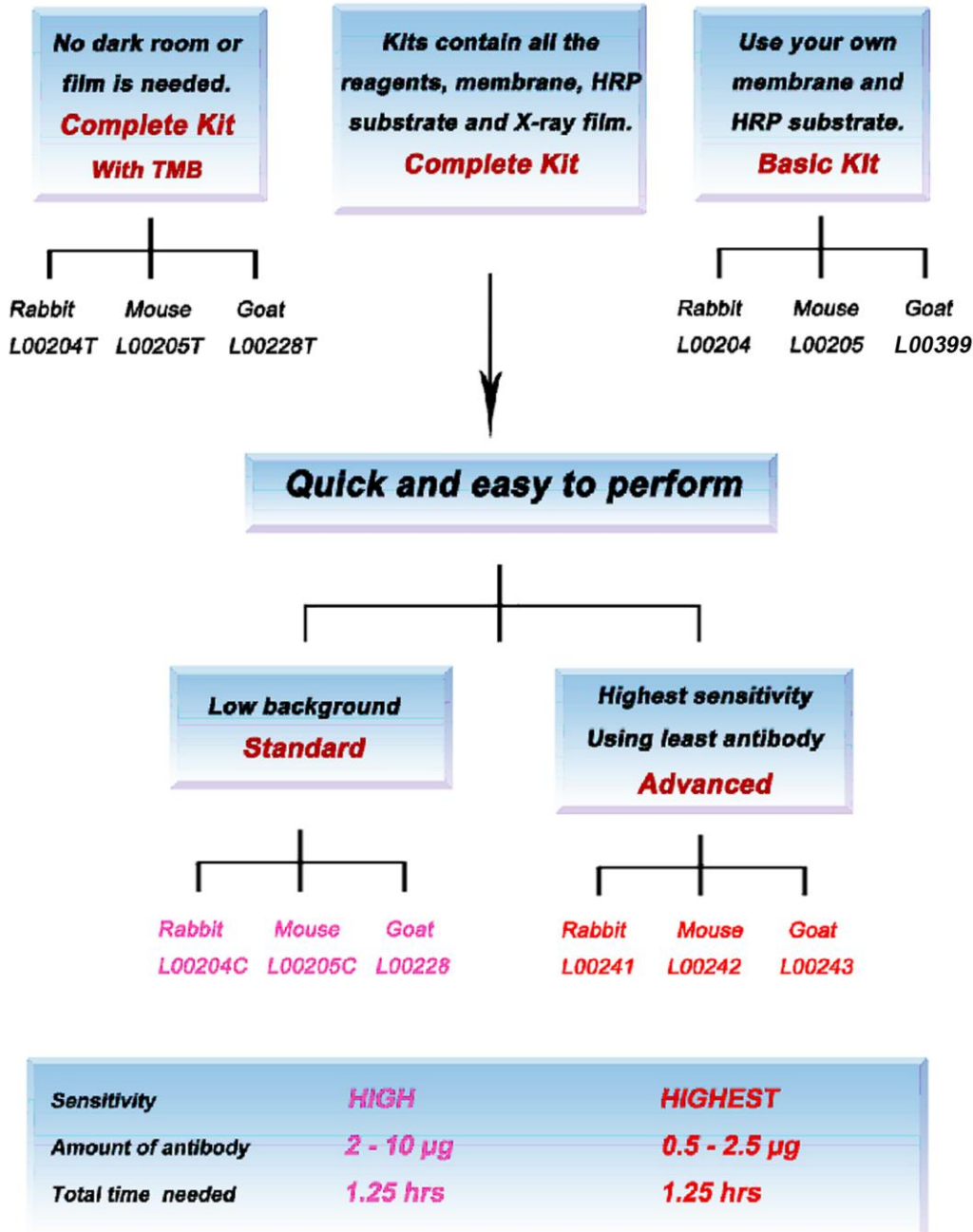


Figure 1. Overview of Western Procedures

II. ONE-HOUR WESTERN KIT SELECTION GUIDE

No Secondary Antibody Is Needed for Any of These Kits



III. KIT CONTENTS

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

Kit Components	Basic Kits	Complete Kits with TMB	Complete Kits	
			Standard	Advanced
Pretreat Solution A	100 ml	100 ml	100 ml	100 ml
Pretreat Solution B	100 ml	100 ml	100 ml	100 ml
WB-1 Solution	1 ml	1 ml	1 ml	1 ml
WB-2 Solution	100 ml	100 ml	100 ml	100 ml
10X Wash Solution	125 ml	125 ml	125 ml	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	X	10 sheets	10 sheets	10 sheets
ChromoSensor™ One Solution TMB Substrate	X	60 ml	X	X
LumiSensor™ Chemiluminescent HRP Substrate*	X	X	2 x 15 ml	10 + 20 ml
High-Sensitivity X-Ray Film (5 x 7 inches)	X	X	20 sheets	20 sheets
Protocol	1	1	1	1

* Standard Complete Kits and Advanced Complete Kits include LumiSensor™ and LumiSensor™ Super Chemiluminescent HRP Substrate, respectively.

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. This solution (GenScript, M01052) is available separately. Customers can also use the GenScript Quick Block Optimization Kit to find the best blocking reagent for their specific projects.

Pretreat A-b	M01052	100 ml
Western Quick Block Optimization Kit	L00278	1 kit

IV. RELATED PRODUCTS

- WestClear™ Nitrocellulose Membrane (0.2 µm) L00224A60
- ChromoSensor™ One Solution TMB Substrate L00222V60
- LumiSensor™ Chemiluminescent HRP Substrate Kit L00221V60
- LumiSensor™ Plus Chemiluminescent HRP Substrate Kit L00225
- LumiSensor™ Super Chemiluminescent HRP Substrate Kit L00354
- 10X Wash Solution MB01011
- Pretreat Solutions (A + B) M01013
- Pretreat A-b M01052
- GenScript Dot Blot Box M00108
- Western Quick Block Optimization Kit L00278
- High-Sensitivity X-Ray Film (5 x 7 inches, 20 sheets) L00358
- Western Blot Box M00100

V. KEY FEATURES

- **Easy to perform:** This kit has fewer and simpler steps than other Western kits.
- **Low background:** The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ series of Chemiluminescent HRP Substrate Kits, and X-ray film, optimized for low background.
- **Reproducible results:** The kit produces highly reproducible results.
- **No additional secondary antibody** is needed.

VI. 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.**

VII. ONE-HOUR 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:

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

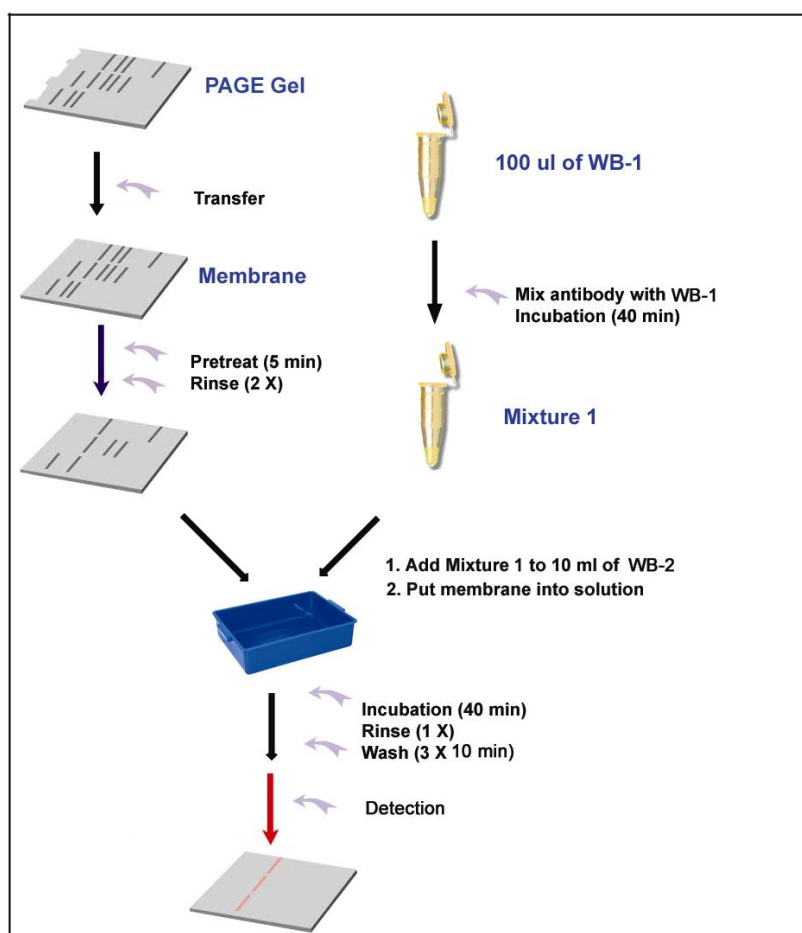
Before use, prepare the following:

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 with WB-1 in a microcentrifuge tube. Vortex mixture 1 gently for a few seconds and centrifuge briefly. Incubate mixture 1 at room temperature (RT) for at least 40 minutes.



Mixture 1 Preparation	L00204, L00205, and L00399	L00204T, L00205T, and L00228T	L00204C, L00205C, and L00228	L00241, L00242, and L00243
WB-1 Solution	20 - 100 μ l	50 - 100 μ l	20 - 100 μ l	10 - 50 μ l
Primary Antibody*	2 - 10 μ g	5 - 10 μ g	2 - 10 μ g	0.5 – 2.5 μ g
Ratio of WB-1: Antibody	10 μ l : 1 μ g	10 μ l : 1 μ g	10 μ l : 1 μ g	20 μ l : 1 μ g
For Antibody without Known Titer	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 5 μ g of Ab with 50 μ l WB-1	Mix 1 μ g of Ab with 20 μ l WB-1

* Refer to manufacturer's recommendations when using appropriate amounts of antibody. With ONE-HOUR Advanced Western Kits, use 1/4 to 1/2 of the recommended amount. For antibodies without known titers, start with 1 μ g for Advanced Western Kits and 5 μ g for other ONE-HOUR Western™ Kits.

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 wash 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

- Add mixture 1 to 10 ml of WB-2 in a Western blot box 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) 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.
- 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. When using TMB substrate, wash the membrane three times for just five 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.**

4-1. Signal Development with Chemiluminescent HRP Substrate

- When using LumiSensor™ HRP Substrate, mix 1.5 ml of reagent A with 1.5 ml of reagent B by vortexing for a few seconds to make the working solution. When using LumiSensor™ Super Chemiluminescent HRP Substrate, mix 1.0 ml of reagent A with 2.0 ml of reagent B by vortexing for a few seconds to make the working solution. 0.05 ml of the working solution is sufficient to cover 1 cm² of membrane. When protected from light, the working solution (A+B) remains stable for several hours at room temperature.

Summary of Working Solution Preparation: 0.05 ml Is Needed per cm² of Membrane

Working Solution Preparation	L00204C, L00205C, and L00228	L00241, L00242, and L00243
Reagent A	1.5 ml	1.0 ml
Reagent B	1.5 ml	2.0 ml
Total Volume	3.0 ml	3.0 ml

- 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.

4-2. Signal Development with TMB Substrate

- a. ChromoSensor™ One Solution TMB Substrate is a ready-to-use working solution, and 0.1 ml is sufficient to cover 1 cm² of membrane. 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 a clean plate and cover it with TMB.
- b. Incubate for 5 to 10 minutes at room temperature until the desired color intensity is reached. Stop the reaction by rinsing the membrane three times for thirty seconds each in 20 ml of deionized water.
- c. Drain off the excess water and transfer the membrane to a piece of paper towel. Air-dry the membrane in a dark place.

VIII. EXAMPLES

Comparison of the two ONE-HOUR Western™ Blot Kits of different sensitivities using monoclonal antibodies:

Two similar blots were processed with the same procedures using different ONE-HOUR Western™ Kits: Standard (L00205C) and Advanced (L00242). 10 µg and 2.5 µg of THE™ Anti-GST Monoclonal Antibody (Mouse) (GenScript, A00865), respectively, were used with these two kits to detect GST protein.

The results are shown in Figure 1.

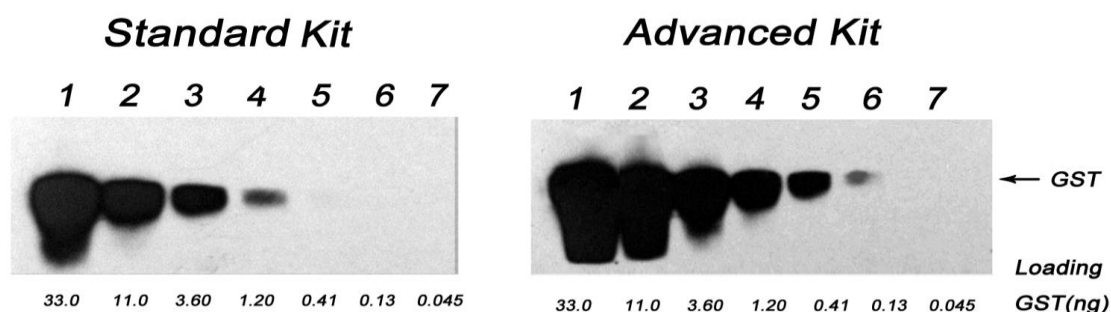


Figure 1. Western blots for the detection of GST protein by ONE-HOUR Western™ using different ONE-HOUR Western™ Kits: Standard (L00205C) and Advanced (L00242). 33.0, 11.0, 3.60, 1.20, 0.41, 0.13 and 0.045 ng of GST protein were loaded into Lanes 1, 2, 3, 4, 5, 6, and 7 respectively.

Comparison of the two ONE-HOUR Western™ Blot Kits of different sensitivities using polyclonal antibodies:

Two similar blots were processed with the same procedures using different ONE-HOUR Western™ Kits: Standard (L00204C) and Advanced (L00241). 10 µg and 2.5 µg of Rabbit Anti-GST-tag Polyclonal Antibody (GenScript, A00097), respectively, were used with the two kits to detect GST protein.

The results are shown in Figure 2.

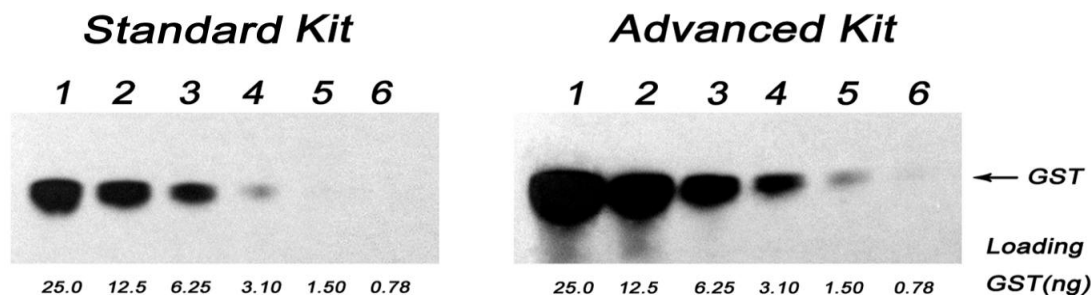


Figure 2. Western blots for the detection of GST protein by ONE-HOUR Western™ using different ONE-HOUR Western™ Kits: Standard (L00204C) and Advanced (L00241). 25.0, 12.5, 6.25, 3.10, 1.50 and 0.78 ng of GST protein were loaded into Lanes 1, 2, 3, 4, 5, and 6 respectively.

IX. 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 pretreat A-b (M01052). Customers can also use the Quick Block Optimization Kit to find the best blocking reagent.
	The wash time is too short.	Adding additional washing steps 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 for background signal to go down 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.

Patent Pending.

For Research Use Only.

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