

One-Step Western™ Complete Kit (Rat)



Technical Manual No. 0214

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I	Description	1
II	Kit Contents	2
III	Applications	2
IV	Key Features	2
V	Storage	2
VI	One-Step Western™ Protocol	3
VII	Examples.....	4
VIII	Troubleshooting	5
IX	Ordering Information	6

I. DESCRIPTION

One-Step Western™ Complete Kit for Rat Primary Antibody (GenScript, L00227) yields a journal-quality Western or Dot blot in just 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 five minutes each. The membrane can then be developed with the HRP substrate included in the kit. The One-Step Western™ procedure is contrasted with a classical Western at right.

The kit contains WestClear™ nitrocellulose membrane (0.2 μm) and LumiSensor™ Chemiluminescent HRP Substrate optimized for best results. WestClear™ nitrocellulose membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit are also available separately.

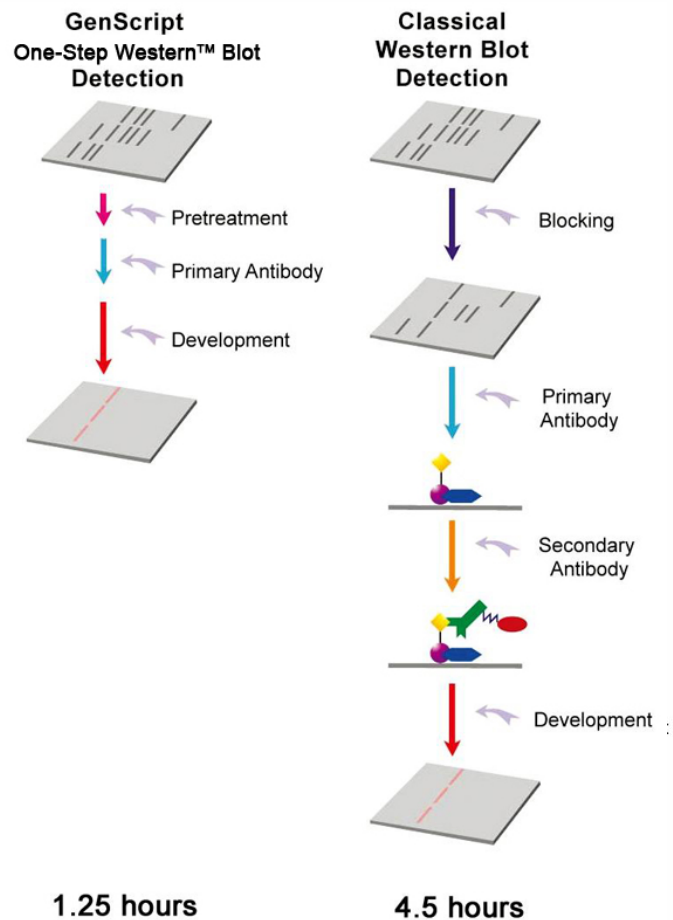


Figure 1. Overview of Western Procedure



II. KIT CONTENTS

Each kit contains enough reagents for 10 mini gel (8 x 8 cm) Western or Dot blot detections.

Kit Components	10 Assays
Pretreat A solution	100 ml
Pretreat B solution	100 ml
WB solution	100 ml
10X wash solution	100 ml
WestClear Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 Sheets
LumiSensor Chemiluminescent HRP Substrate	2 x 30 ml
Protocol	1

III. APPLICATIONS

The One-Step Western™ Complete Blot Kit has applications that include the following:

- Protein (antigen) detection
- Confirmation of protein expression
- Antibody/antigen titration

IV. KEY FEATURES

- ◆ Easy to perform: Fewer steps mean fewer chances for human error.
- ◆ Low background: The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit, optimized for low background.
- ◆ High sensitivity: The kit's sensitivity is comparable with or better 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.
- ◆ Needs far less optimization than the classical method.

V. STORAGE

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

VI. ONE-STEP WESTERN™ PROTOCOL

This procedure is optimized for a sheet of 7.5 x 8 cm membrane. The volumes of reagents can be scaled up or down according to the size of the membrane to be used.

**Reagents needed but not provided in the kit:**

Rat primary antibodies. Affinity-purified antibodies are preferred.

Before use, prepare the following:

1. Gently invert each solution bottle several times to mix well.
2. Dilute 10 ml of 10X wash solution with 90 ml of distilled or filtered water to make a 1X wash solution, use 14 ml for each rinse or 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 ddH₂O to 1X and store it at 4°C.
3. Mix 10 ml of pretreat A solution with 10 ml of pretreat B solution in a plastic container such as Western Wash Box (GenScript, M00100) to make the pretreat solution mixture.
4. Add 5 to 20 µg of primary antibody to 10 ml of corresponding WB solution and mix well. This mixture can be recovered and reused up to three times, depending on the antibody concentration. However, carryover contamination may occur and the antibody concentration change may cause variations in results. None of the other reagents are reusable.

Western/Dot blot procedure:**Transferring or spotting proteins to membrane**

For Dot blots, spot the protein samples directly onto the membrane. For Western blots, float the nitrocellulose membrane in deionized water until it is completely wet, then soak it in transfer buffer until use. Follow standard transfer procedures.

Western or Dot blot

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

1. Incubate the membrane in 20 ml of the pretreat solution mixture (mixture of pretreat A and pretreat B) for 5 min at room temperature on a shaker. Longer incubation is not necessary and do not incubate the membrane in pretreatment solution for more than 15 minutes. After incubation, rinse the membrane with 14 ml of 1X wash solution two times.
2. Incubate the membrane from step 1 on a shaker with 10 ml of WB solution containing the primary antibody for 40 minutes at room temperature.
3. Rinse the membrane once with 14 ml of 1X wash solution, then wash the membrane three times on a shaker for five minutes each with 14 ml of 1X wash solution. Use a clean container for each rinse and wash step to avoid carryover contamination and to reduce background.
4. (Optional) Wash the membrane one more time for five minutes with 1X wash solution to further decrease background.

Signal development

Develop the membrane from the Western or Dot blot with the LumiSensor™ Chemiluminescent HRP Substrate provided in the kit.

1. Mix 3 ml of reagent A with 3 ml of reagent B by vortexing for a few seconds to make the working solution (use 0.1 ml of the working solution per cm² of membrane). The working solution should be warmed up to room temperature before use. The working solution is stable for several hours at room temperature when protected from light.
2. Drain off the excess wash solution from the membrane by holding it vertically with forceps and touching the edge against a tissue. Place the membrane on clean, flat surface, and cover the membrane with the working solution.
3. Incubate for three minutes at room temperature. Place the membrane on a clean tissue. Use a soft clean tissue to remove excess working solution. Wrap the membrane in a clean piece of plastic film.



4. Expose to a sheet of film for 30 seconds and develop the film. Repeat this step with different exposure times for best results.

VII. EXAMPLES

Western blot detection of housekeeping protein α -Tubulin using rat antibody:

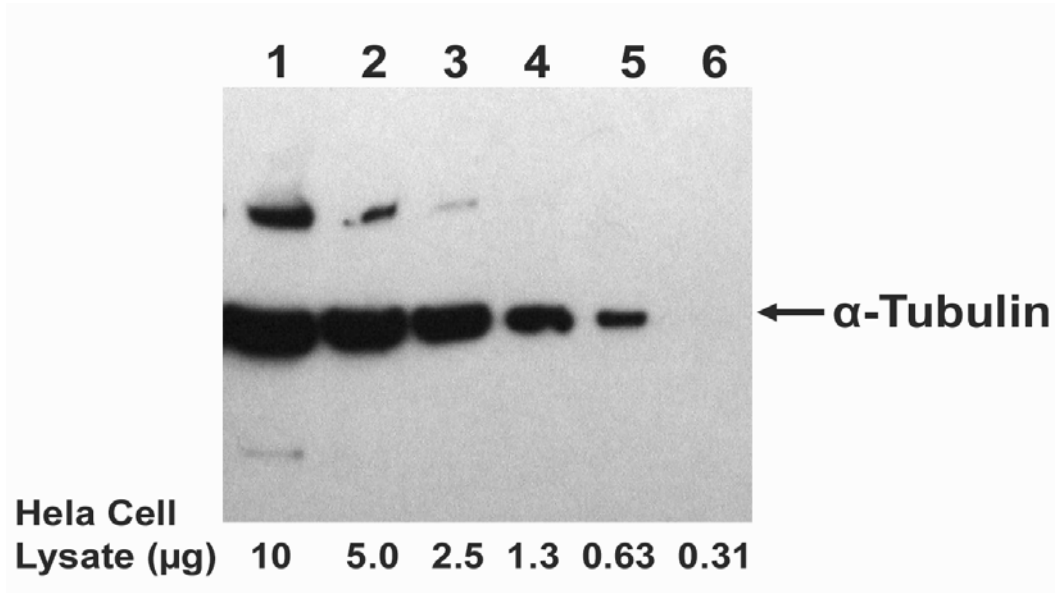


Figure 2. Western blot detection of α -Tubulin using One-Step Western™ Complete Blot Kit (Rat) (GenScript, L00227) and rat α -Tubulin antibody (GeneTex, GTX76511). 10 μ g, 5.0 μ g, 2.5 μ g, 1.25 μ g, 0.62 μ g, and 0.31 μ g of HeLa cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, and Lane 6, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

α -Tubulin from 0.63 μ g of HeLa cell lysate (Lane 5) can be cleanly detected using the kit.



VIII. TROUBLESHOOTING

Use the table below to solve and avoid common problems.

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 gel.
	There is poor specific binding activity of the primary antibody.	Use purified antibodies
	The primary antibody is too diluted.	Increase the concentration of the primary antibody.
	The incubation time is too short or the reagent is too cold.	In most cases, a 40-minute incubation at room temperature is enough. However, if the WB solution has not been allowed to come to room temperature before the blot, then use a longer incubation time.
There is high background and/or non-specific bands on the blot.	The primary antibody shows non-specific binding or cross-reactivity.	Select a highly specific primary antibody. Purified antibodies are preferred.
	Too much primary antibody has been added to the One-Step Western™ Blot solution.	Reduce the concentration of primary antibody in the WB solution. Optimize the antibody concentration using Dot-blot. Using diluted WB solution can also decrease background. WB solution can be diluted with PBS containing 0.1% Tween-20.
	The wash time is too short.	Adding an additional wash step after primary antibody binding (in WB) can further decrease background.
	The signal development time is too long.	Reduce the development time.
	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.
	The signal development reagent too sensitive.	Use chromogenic development reagents, such as TMB, which is less sensitive and produces lower background than the chemiluminescent reagent.



IX. ORDERING INFORMATION

One-Step Western™ Complete Kit: L00227 for rat primary antibody

Patent Pending.

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