One-Step Western™ Blot Kit

Technical Manual No. 0184 **Version 20080325**

I. DESCRIPTION

One-Step Western™ Blot Kit is one of GenScript newly introduced innovative products. Using a breakthrough immuno-detection technology (patent pending), the kit was specifically developed for fast Western blot and Dot blot. Instead of using the classical three-step procedure (blocking, primary antibody-binding and secondary antibody-binding) that can take about 4.5 hrs, this kit allows you to perform Western blot or Dot blot within an hour. After transferring proteins from a gel to the membrane (or using dot blot membrane), you simply incubate the membrane in pretreat solution mixture for 5 minutes followed by incubation in WB solution with the primary antibody for 40 min, and wash the membrane three times for 5 min each, you are now ready to develop the blots. One-Step Western™ Blot Kit is a horse radish peroxidase (HRP) based kit, one can use either Chemiluminescent substrate or 3, 3'-Diaminobenzidine (DAB) or TMB to develop the blots. A comparison of GenScript One-Step Western™ blot procedure with the classical three-step procedure is shown in Figure 1.

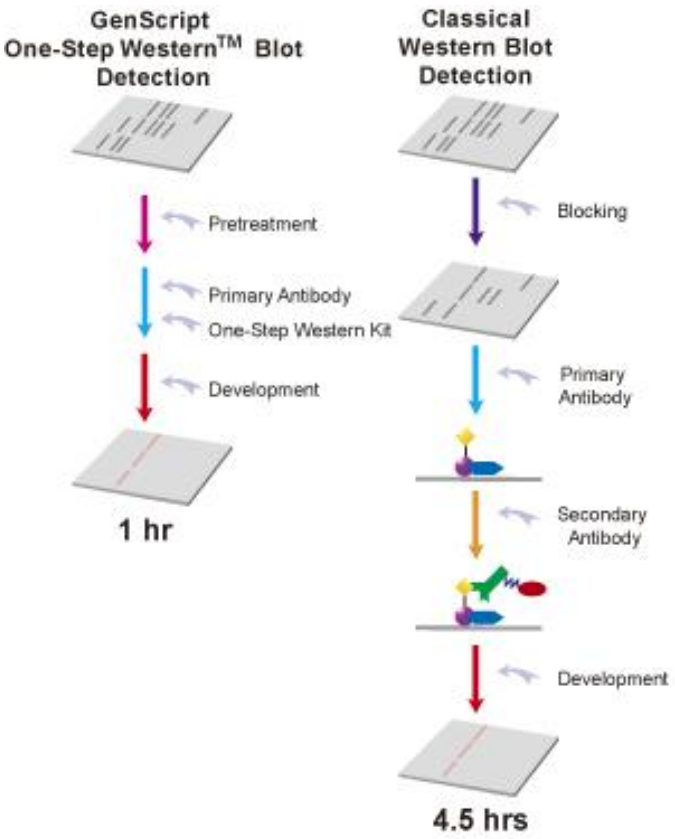

Figure 1. Overview of Western Procedure

Currently GenScript provides two different basic One-Step Western™ Blot Kits. One is to be used with rabbit primary antibody for Western blot (or Dot blot), and the other with mouse primary antibody for Western blot (or Dot blot).

To obtain best results and reduce variations caused by using different membranes and detection reagents, it is strongly recommended that the following reagents be used with One-Step Western™ Blot Kit:

GenScript WestClear™ nitrocellulose membrane (L00224A60)
GenScript LumiSensor™ Chemiluminescent HRP Substrate Kit (L00221V60)

II. KIT CONTENTS

Two different kinds of One-Step Western™ Blot Kits are available. L00204 is for detection using rabbit primary antibody (whole molecule), and L00205 is for using mouse primary antibody. Each kit contains enough reagents for 10 mini gel (8 x 8 cm) Western blot (or Dot blot) detections.

These kits are not recommended for antibodies against phosphoprotein.

Kit Components	10 Assays
Pretreat A solution	100 ml
Pretreat B solution	100 ml
WB solution	100 ml
10X Wash solution	125 ml
Dot Blot Box	1
Protocol	1

Product	Catalog No.
Kit For Rabbit Primary Antibody	L00204
Kit For Mouse Primary Antibody	L00205

III. APPLICATIONS

One-Step Western™ Blot Kit enables the fast Western blot or Dot blot for the applications such as:

- Detecting proteins (antigens)
- Confirmation of protein expression
- Titrating antibody or antigen

IV. KEY FEATURES

- Easy to perform: simple and rapid procedure to perform Western blot or Dot blot in one hour.
- High sensitivity: the sensitivity using this kit is comparable with or better than that obtained from the classical 4. 5 hr procedure (depending on the quality and amount of antibodies used).
- Reproducible results: the kit produces highly reproducible results.
- No secondary antibody is needed.
- Much less optimization is needed compared with the classical method.

V. STORAGE

Don't freeze the kit or any components, store the kit at 4°C (stable for 6 months).

VI. ONE-STEP WESTERN™ BLOT KIT PROTOCOL

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

Reagents needed but not provided in the kit:

1. Primary antibodies. Purified monoclonal or affinity-purified polyclonal antibodies are preferred. The rabbit polyclonal antibody should be the whole molecule, Fab fraction gives a significantly low signal. These kits are not recommended for use with antibodies against phosphoprotein.
2. Chemiluminescent substrate (e.g. GenScript LumiSensor™ Chemiluminescent Substrate Kit L00221V60) or chromogenic substrate (e.g. GenScript ChromoSensor™ One-Solution TMB Substrate L00222V60).

Before use, prepare the following:

1. Gently invert each solution bottle several times to mix well.
2. Dilute 12.5 ml of 10X wash solution with 112.5 ml of distilled or filtered water to make a 1X wash solution, use 20 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 ddH_2O 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. Make this solution just before the protein transfer from gel to membrane is complete. Always use fresh mixture.

Antibody Concentration Titration Test

Due to the varying affinity and specificity of antibodies and the differing sensitivity of signal detection system (HRP substrate and film, etc.) 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, use 2 ml of fresh pretreat solution mixture (pretreat A plus pretreat B) for pretreatment of the strips.
3. Prepare and use these three different WB solutions containing different amounts of primary antibody for titration test: a). Add 1 µg of primary antibody to 1 ml of WB solution and mix well. b). Add 0.5 µg of primary antibody to 1 ml of WB solution and mix well. c). Add 0.2 µg of primary antibody to 1 ml of 50% WB solution (diluted with PBS or PBS+ 0.1% Tween 20) and mix well.
4. Process the membrane as described in the following procedure and select the best antibody and WB ratio for future western blot analyses.

Western or 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 procedure for transferring.

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 the pretreat solution mixture on a shaker for five minutes at room temperature. Do not incubate the membrane for more than 15 minutes. After incubation, rinse the membrane with 20 ml of 1X wash solution two times.
2. Add 2 to 10 µg of primary antibody (as determined by titration test) to 10 ml of WB solution (or diluted WB) and mix well. Incubate the membrane from step 1 on a shaker with this solution for 40 minutes at room temperature.
3. Rinse the membrane once with 20 ml of 1X wash solution, then wash the membrane on a shaker three times for five minutes each with 20 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 with 1X wash solution for five minutes to further decrease background.
5. Develop the blot with Chemiluminescent, or chromogenic substrate according to the manufacturer's instructions. It is strongly recommended that GenScript LumiSensor™ Chemiluminescent HRP Substrate Kit (L00221V60) or ChromoSensor™ One-Solution TMB Substrate (L00222V60) be used for signal development.

VII. EXAMPLES USING THE KIT

Comparison of One-Step Western™ blot detection with classical Western blot detection.
One-Step Western™ blot detection was compared with classical Western blot detection of α-Tubulin protein from Hela cell lysate. Serially diluted Hela cell lysate samples were Western blotted to GenScript WestClear™ nitrocellulose membrane (L00224) after SDS-PAGE. The membrane was then cut into two halves and processed with different procedures using α-Tubulin monoclonal antibody (abcam, ab7291): One-step Western™ blot detection (1 hr, right panel of Figure 2) and classical Western-blot detection (4.5 hours, left panel of Figure 2).

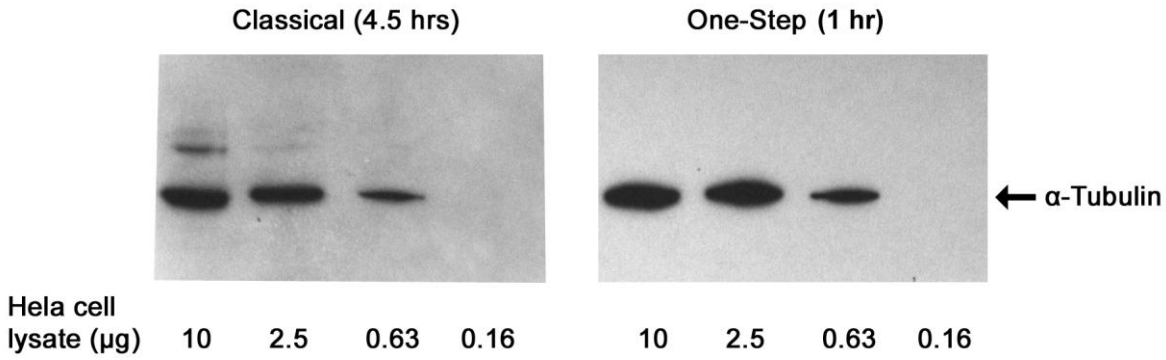

Figure 2. Western blots for the detection of α-Tubulin protein from Hela cell lysate by both classical western and One-Step western™ (using kit L00205). 10 µg, 2.5 µg, 0.63 µg, and 0.16 µg of Hela cell lysate (BD Biosciences, 611449) were loaded in different lanes as shown in the figure. The classical western blot was developed with ECL system (GE Healthcare, RPN2109). The One-Step western blot was developed with GenScript LumiSensor™ Chemiluminescent HRP Substrate (L00221V60).

VIII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Signal weak or invisible	Too little protein is loaded.	Load more protein(s) onto the SDS-PAGE gel.
	Poor transfer efficiency.	Optimize transfer time and/or the electrical current. Make sure that there are no air bubbles between the membrane and gel.
	Primary antibody diluted too much.	Increase the concentration of the primary antibody. Dot blots with serially diluted samples (from 1 µg/µl to 1 ng/µl) and serially diluted primary antibody is recommended to optimize primary antibody concentration.
	Incubation time too short.	In most cases, 40-minute incubation at room temperature is sufficient. However, if the primary has low affinity, longer incubation time (1 hour to several hours) is needed.
High background, non-specific bands on blot	Membrane or HRP substrate is not optimal.	Use GenScript WestClear™ nitrocellulose membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit.
	Non-specific binding /cross-reactivity of primary antibody.	Select highly specific primary antibody. Purified monoclonal or affinity-purified polyclonal primary antibodies are preferred.
	Too much primary antibody added to the One-Step WB solution.	Reduce the concentration of primary antibody added to the WB solution. Optimize the antibody concentration by antibody concentration titration.
	Wash time too short.	Adding one more wash with 1X Wash solution after primary antibody binding always decreases background.
	Signal development time too long.	Reduce the development time.
	Contaminated reagents or equipment.	Use a clean container every time you change solution for rinse and wash. Wear gloves and use clean forceps to handle membranes.

IX. ORDER INFORMATION

One-Step Western™ Kit: L00204 for rabbit primary antibody.
L00205 for mouse primary antibody.

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