One-Step Western™ Complete Kit

Technical Manual No. 0202 **Version 03262008**

I. DESCRIPTION

The One-Step Western™ Complete Kit 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 (L00224A60), LumiSensor™ Chemiluminescent HRP Substrate Kit (L00221V60) and Five-Slot Dot Blot Box (M00108) are also available separately.

However, the kit is not recommended for use with antibodies against phosphoprotein.

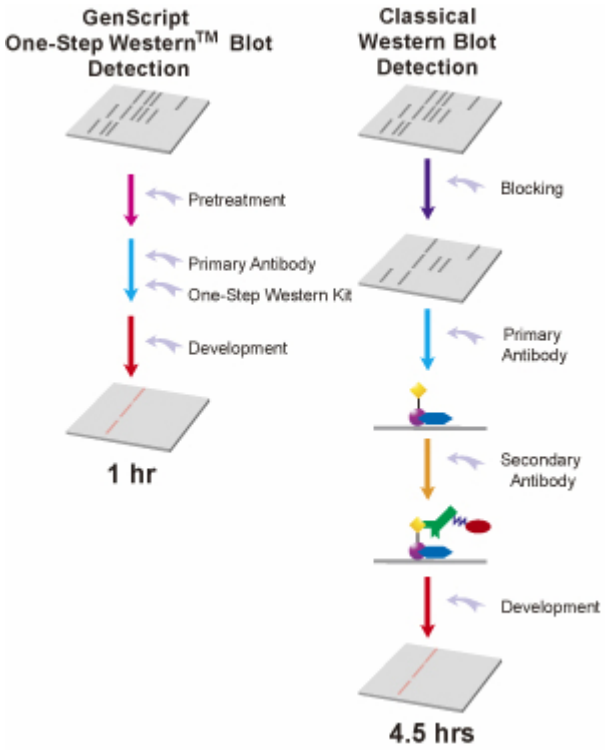

Figure 1. Overview of Western Procedure

II. KIT CONTENTS

The kits contain enough reagents for 10 mini gel (7.5 x 8 cm) Western blots, respectively.

Kit Components	10 Assays L00204C & L00205C
Pretreat A solution	100 ml
Pretreat B solution	100 ml
WB solution	100 ml
10X wash solution	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 sheets
LumiSensor™ Chemiluminescent HRP Substrate	2 x 30 ml
Dot Blot Box	1
Protocol	1

III. APPLICATIONS

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

- Protein (antigen) detection
- Confirmation of protein expression
- Titration of antibodies and antigens

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.
- 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 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^2 membrane. The volumes of the reagents can be scaled up or down according to the size of the membrane used.

Reagents not provided:

Primary antibodies: Purified monoclonal or affinity-purified polyclonal antibodies are preferred. The rabbit polyclonal antibody should be whole-molecule, Fab fractions give a significantly lower signal. These kits are not recommended for use with antibodies against phosphoprotein.

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 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, 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.

Signal Development

Develop the membrane from the Western blot or Dot blot after the final wash. Use 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 cm^2 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 the membrane 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 it. Repeat this step with different exposure times to get the best results.

VII. EXAMPLES

The One-Step Western™ is compared to a classical three-step Western below.

One-Step Western™ Blot detection was compared with a classical western blot detection of α-Tubulin protein from *Hela* cell lysate. Serially diluted *Hela* cell lysate samples were Western-blotted to WestClear™ nitrocellulose membrane after SDS-PAGE. The membrane was then cut into two halves and processed with different procedures using α-Tubulin Monoclonal Antibody (abcam, ab7291): Classical Western blot detection (4.5 hours, left panel of Figure 2), and One-Step Western™ Blot detection (one hour, right panel of Figure 2).

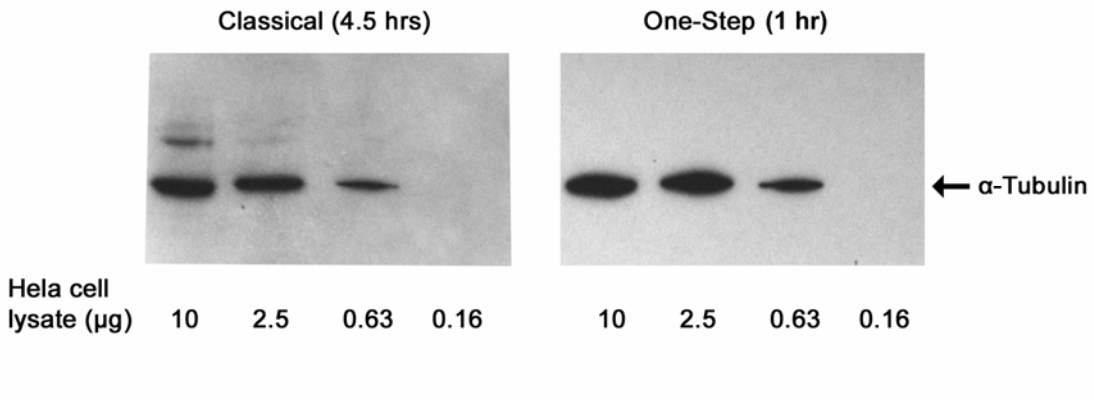

Figure 2. Western blots for the detection of α-Tubulin protein from *Hela* cell lysate by both classical Western and One-Step Western™ Complete Kit (Mouse) (GenScript, L00205C). 10 µg, 2.5 µg, 0.63 µg, and 0.16 µg of *Hela* cell lysate (BD Biosciences, 611449) were loaded in different lanes as shown in the figure. The classical Western blot was developed with ECL system (GE Healthcare, RPN2109). The One-Step Western Blot was developed with LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

Dot blot detection of IL-8 protein.

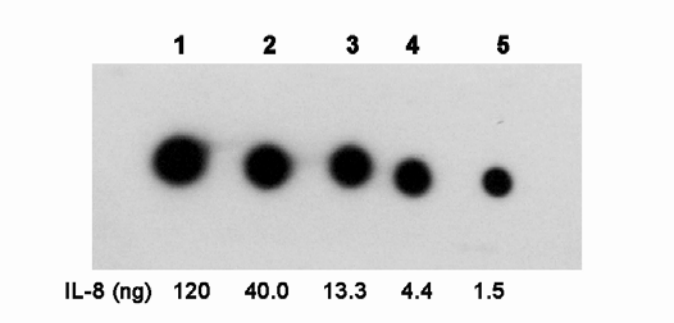

Figure 3. Dot blot for the detection of IL-8 protein using the One-Step Western™ Complete Kit (Mouse) (GenScript, L00205C) and Mouse IL-8 Antibody (Endogen, M801). 120 ng, 40.0 ng, 13.3 ng, 7.4 ng, 4.4 ng and 1.5 ng of IL-8 protein were spotted on the membrane. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

Western blot detection of housekeeping protein α-Tubulin using polyclonal antibody.

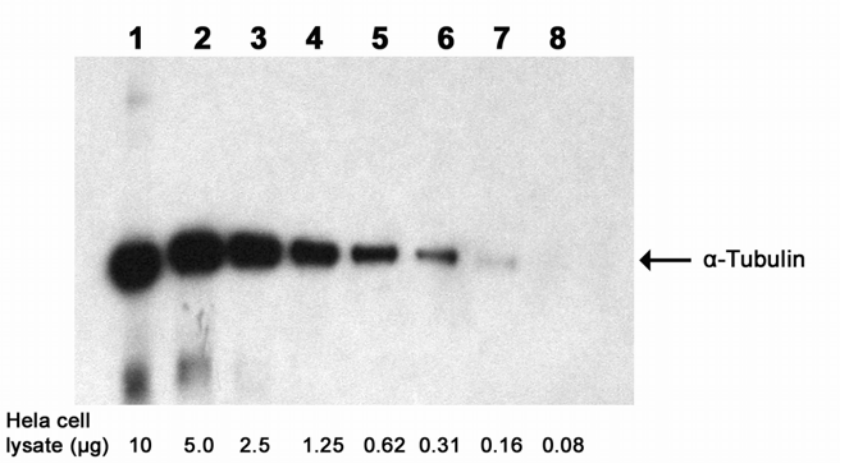

Figure 4. Western blot for the detection of α-Tubulin using the One-Step Western™ Complete Kit (Rabbit) (GenScript, L00204C) and α-Tubulin Polyclonal Antibody (abcam, ab4074). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg, 0.31 µg, 0.16 µg, and 0.08 µg of *Hela* cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, Lane 7, and Lane 8, respectively. The blot was developed with LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

Western blot detection of housekeeping protein GAPDH using monoclonal antibody.

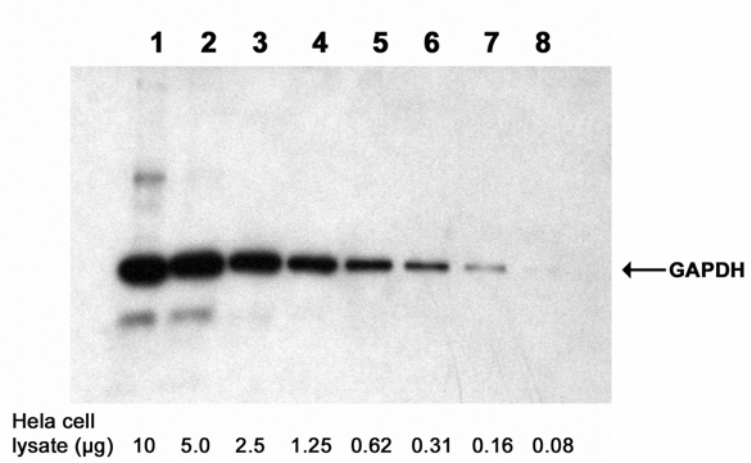

Figure 5. Western blot for the detection of GAPDH using the One-Step Western™ Complete Kit (Mouse) (GenScript, L00205C) and Mouse GAPDH Antibody (abcam, ab8245). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg, 0.31 µg, 0.16 µg and 0.08 µg of *Hela* cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, Lane 7, and Lane 8, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate included in 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 has been 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.
	The primary antibody shows poor specific binding activity.	Use purified monoclonal or affinity-purified polyclonal primary antibodies.
	The primary antibody is too diluted.	Increase the concentration of the primary antibody.
	The incubation time is too short.	In most cases, a 40-minute incubation at room temperature is enough. However, if the primary has low affinity, longer incubation time (1 hour to several hours) is needed.
There is high background and/or non-specific bands on the blot.	There is non-specific cross-reactivity of primary antibody.	Select highly specific primary antibodies. Purified monoclonal or affinity-purified polyclonal primary antibodies are preferred.
	Too much primary antibody has been 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. Using diluted WB solution can also decrease background. WB solution can be diluted with PBS or PBS containing 0.1% Tween-20.
	The wash time is too short.	Adding one more wash with 1X wash solution always decreases background.
	The signal development time is too long.	Reduce the development time.
	The equipment or reagents are contaminated.	Use a clean container every time you change solution for the rinse and wash steps. Wear gloves and use clean forceps to handle the membranes.
	The signal development reagent is too sensitive.	Use chromogenic development reagents, such as DAB or TMB, which is less sensitive and produces lower background than chemiluminescent reagents.

IX. ORDERING INFORMATION

One-Step Western™ Complete Kit: L00204C for rabbit primary antibody.
L00205C for mouse primary antibody.

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Patent Pending.

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