One-Step Western™ Complete Kit (Chicken)

Technical Manual No. 0206 **Version 03312008**

I. DESCRIPTION

One-Step Western™ Complete Kit for chicken primary antibody (GenScript, L00226) 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.

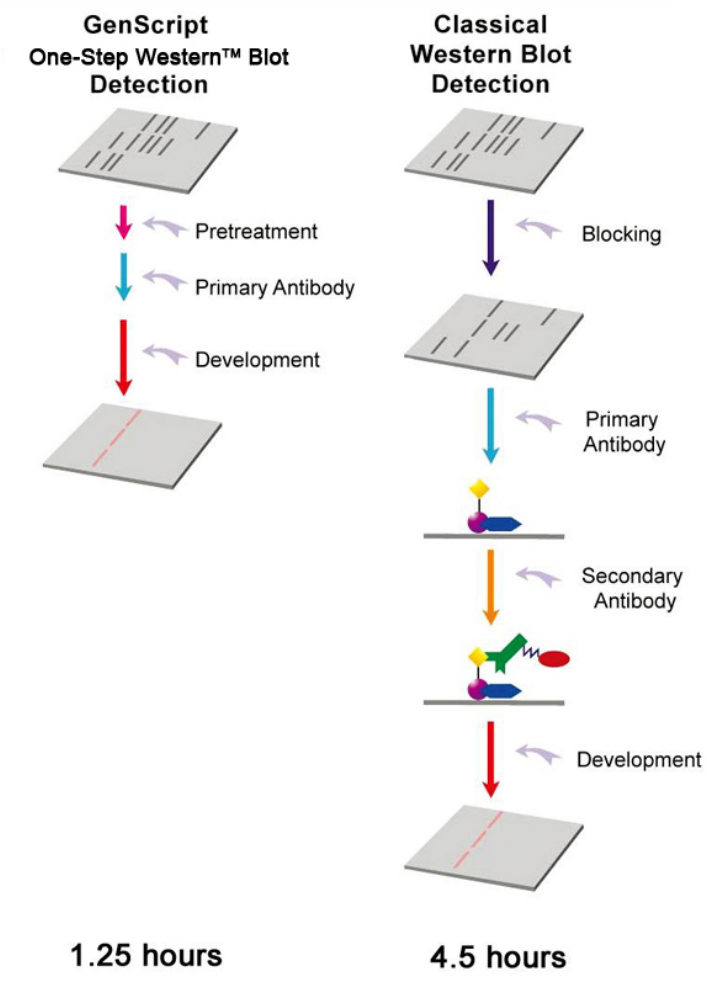

Figure 1. Overview of Western Procedure

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
- 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 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:
Chicken 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 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.
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 or Dot blot procedure:

Transferring/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

1. Do not wash the membrane after transferring the proteins from the gel. Proceed directly to the steps below. Incubate the membrane in 20 ml of the pretreat solution mixture (mixture of pretreat A and pretreat B) on a shaker for five min at room temperature. Do not incubate the membrane 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 with 10 ml of WB solution containing the appropriate primary antibody on a shaker for 40 minutes at room temperature.
3. Rinse the membrane once with 14 ml of 1X wash solution. Then wash the membrane on a shaker three times 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 with 1X wash solution for five minutes 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 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 it vertically with forceps and touching the edge against a tissue. Place the membrane on clean, flat surface, and cover it with 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 up the membrane with 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 β-Actin using chicken antibody:

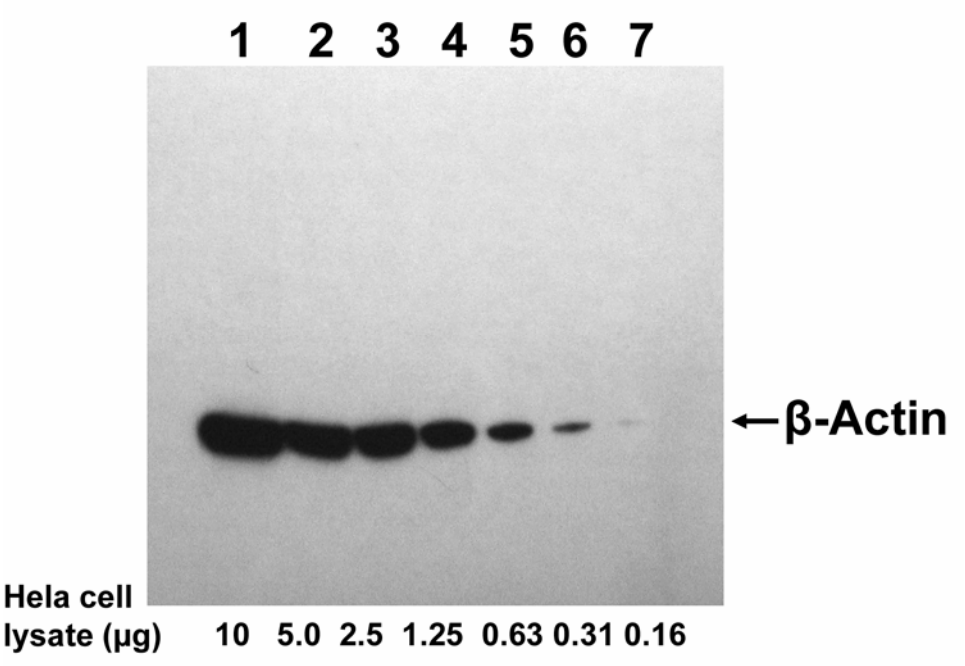

Figure 2. Western blot for the detection of β-Actin using One-Step Complete Western™ Blot Kit (Chicken) (GenScript, L00226) and chicken β-Actin antibody (GeneTex, GTX14001). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg, 0.31 µg, and 0.16 µg of Hela cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, Lane 6, and Lane 7, respectively. The blot was developed with the LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

β-Actin from 0.31 µg of Hela cell lysate (Lane 6) can be detected cleanly using the kit. A weak band from even 0.16 µg of Hela cell lysate (Lane 7) can also be seen.

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 primary 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 primary 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 a Dot-blot.
	The wash time is too short.	Adding an additional wash step after the 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 is 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: L00226 for chicken primary antibody.

Patent Pending.

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