One-Step Western™ Complete Kit (Goat or Sheep)

Technical Manual No. 0215 **Version 03312008**

I. DESCRIPTION

One-Step Western™ Complete Kit (GenScript, L00228, for goat or sheep primary antibody) is a complete kit containing all the necessary reagents, buffers, nitrocellulose membrane and HRP substrate for performing Western Blot or Dot Blot using goat or sheep primary antibody. 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 using the HRP substrate included in the kit. Western blot procedure with the classical three-step procedure is shown in figure 1.

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.

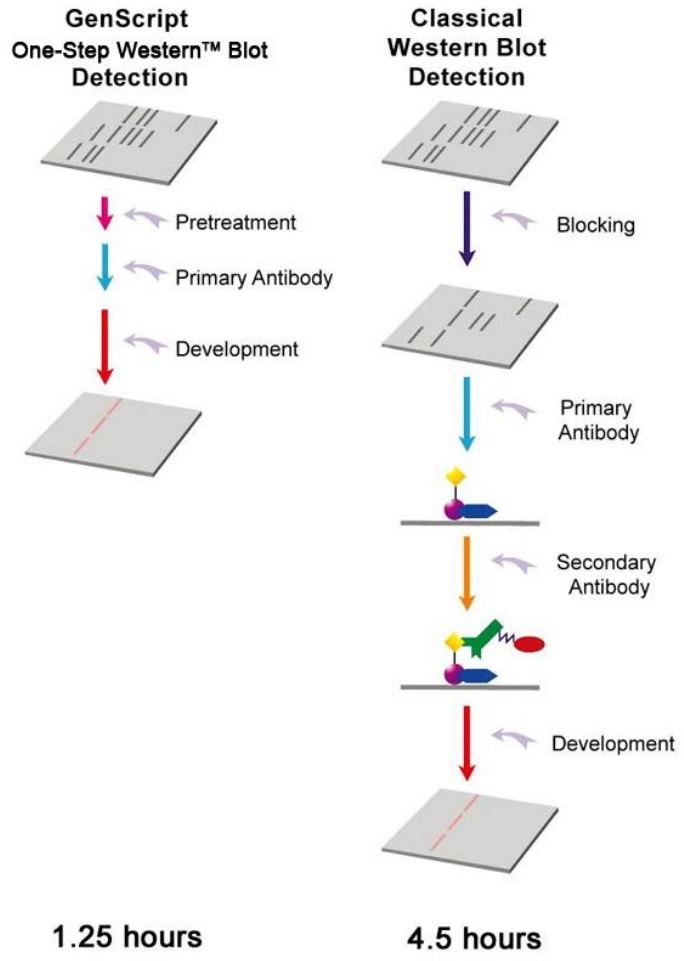

Figure 1. Overview of Western Procedure

II. KIT CONTENTS

Each kit contains enough reagents for 10 mini gel (8 x 7.5 cm) Western blot (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

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

- Detecting proteins (antigens).
- Checking protein expression.
- Titrating antibody or antigen.

IV. KEY FEATURES

Easy to perform: simple and rapid procedure to perform Western blot or Dot blot in an hour.

- Low background: the kit also contains WestClear™ Nitrocellulose Membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit that are optimized to give low background.
- 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, only the amount of primary antibody may need optimization.

V. STORAGE

Store WestClear™ Nitrocellulose Membrane at room temperature. Store the rest of the kit at 4°C (stable for 3 months). **Don't freeze the kit or any component**.

VI. ONE-STEP WESTERN™ KIT PROTOCOL

This procedure is optimized for a sheet of 8 x 7.5 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:

Goat or sheep primary antibodies (whole molecule). Affinity-purified antibodies are preferred.

Before use, prepare the following:

1. Warm all solutions to room temperature.
2. Mix 10 ml of pretreat A solution with 10 ml of pretreat B solution to make the pretreat solution mixture in a plastic container (for example, the cover from a pipette tip box). Pretreat A solution may form a gel by itself during storage, the gel will melt by warming up slightly. Mix by gently inverting the bottle several times before use.
3. Add 5-20 µg (or following manufacturer's recommendations) of primary antibodies to 10 ml of corresponding WB solution and mix well. This mixture can be recovered and reused up to three times depending on the concentration of the antibody. However, variations may arise due to antibody concentration change and carry-over contamination. Other reagents (pretreat A and B, wash solution) are not reusable.
4. Dilute 10 ml of 10X wash solution with 90 ml of distilled or filtered water to make a 1X wash solution, use 14 ml of 1X wash solution for each rinse or wash.

Western blot or Dot blot procedure:

Transferring or Spotting Proteins to Membrane

For Dot blot, spot protein samples directly on the membrane. For Western blot, float nitrocellulose membrane in deionized water until completely wet, then soak in transfer buffer until use. Follow standard procedure for transferring.

Western blot or Dot blot

The Dot blot membrane or the membrane after the transfer step is directly processed without any wash.

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 min. 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 for 40 min at room temperature with shaking.
3. Rinse the membrane once with 14 ml of 1X wash solution, then wash the membrane three times (5 min each time) with 14 ml of 1X wash solution on a shaker. Use a clean container every time you change solutions for rinse and wash steps to avoid carryover contamination and to reduce background.
4. (Optional) Wash the membrane one more time with 1X wash solution (5 min each time) to further decrease background.

Signal Development

The membrane from Western blot or Dot blot after the final wash will be developed with the LumiSensor™ Chemiluminescent HRP Substrate.

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 3 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 60 seconds and develop the film. Repeat this step with different exposure time to get the best results.

VII. EXAMPLES USING THE KIT

A. Western blot detection of GAPDH using goat antibody.

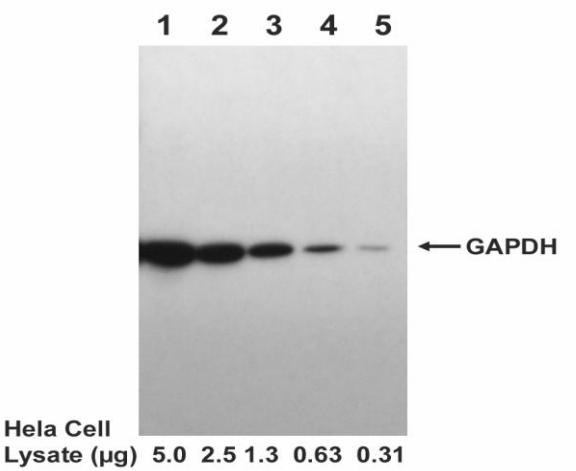

Figure 2. Western blot for the detection of housekeeping protein GAPDH using One-Step Western™ Complete Kit (GenScript, L00228) and Goat Anti-GAPDH Polyclonal Antibody (GenScript, A00191). 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg and 0.31 µg of Hela cell lysate (BD Biosciences, 611449) were loaded in lane 1-5, respectively. The blot was developed with LumiSensor™ Chemiluminescent HRP Substrate included in the kit.

GAPDH protein from 0.31 µg of Hela cell lysate (Lane 5) can be cleanly detected using the kit.

B. Western blot detection of Histone H3 protein using sheep antibody.

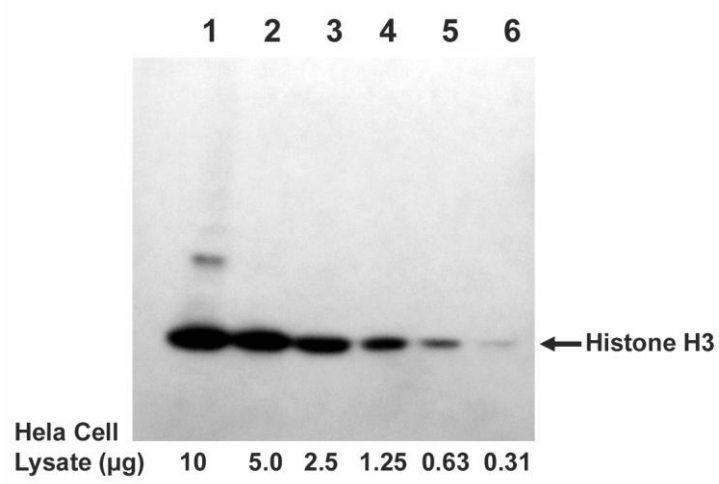

Figure 2. Western blot for the detection of Histone H3 protein using One-Step Western™ Complete Kit (GenScript, L00228) and sheep Histone H3 antibody (abcam, ab7834). 10 µg, 5.0 µg, 2.5 µg, 1.25 µg, 0.62 µg and 0.31 µg of Hela cell lysate (BD Biosciences, 611449) were loaded in Lane 1-6, respectively. The blot was developed with LumiSensor™ Chemiluminescent HRP Substrate included in the kit.
Histone H3 protein from 0.31 µg of Hela cell lysate (Lane 6) can be cleanly detected using the kit.

VIII. TROUBLESHOOTING

The table below is guideline for 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.
	Poor specific binding activity of the primary antibody.	Use purified antibodies.
	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 or the reagent not warmed up properly.	In most cases, 40-minute incubation at room temperature is enough. However, if the WB solution stored in a refrigerator/freezer is used before pre-warmed up to RT, longer incubation time is needed.
High background, non-specific bands on blot	Non-specific binding /cross-reactivity of primary antibody.	Select highly specific primary antibody. Purified antibodies are preferred.
	Too much primary antibody added to the One-Step Western™ blot solution.	Reduce the concentration of primary antibody added to the WB solution. Optimize the antibody concentration using Dot blot.
	Wash time too short.	Adding additional wash after primary antibody (in WB) binding can further decrease 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.
	Signal development reagent too sensitive.	Use chromogenic development reagents, such as TMB, which is less sensitive and produce lower background than chemiluminescent reagent.

IX. ORDER INFORMATION

One-Step Western™ Complete Kit: Cat. No. L00228 for goat or sheep primary antibody.

Patent Pending.

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