One-Step Western™ Trx Detection Kit

Technical Manual No. 0208 **Version 03282008**

I. DESCRIPTION

Based on GenScript One-Step Western™ Detection technology, the One-Step Western™ Trx Detection Kit provides a journal-quality western blot, revealing Thioredoxin (Trx) protein or proteins tagged with Trx. 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 for 40 minutes, and lastly, wash three times for five minutes each. The membrane can now be developed with the HRP substrate included in the kit. The One-Step Western™ procedure is contrasted with a classical Western at right.

This kit can detect Trx alone or fused to any part of another protein, N-terminus, C-terminus, or internal.

The One-Step Western™ Trx Detection Kit contains all the necessary reagents, buffers, nitrocellulose membrane and HRP substrate for performing Western or Dot blot. Neither a primary antibody nor a secondary antibody is needed. The kit includes our highly sensitive chemiluminescent substrate for HRP signal development.

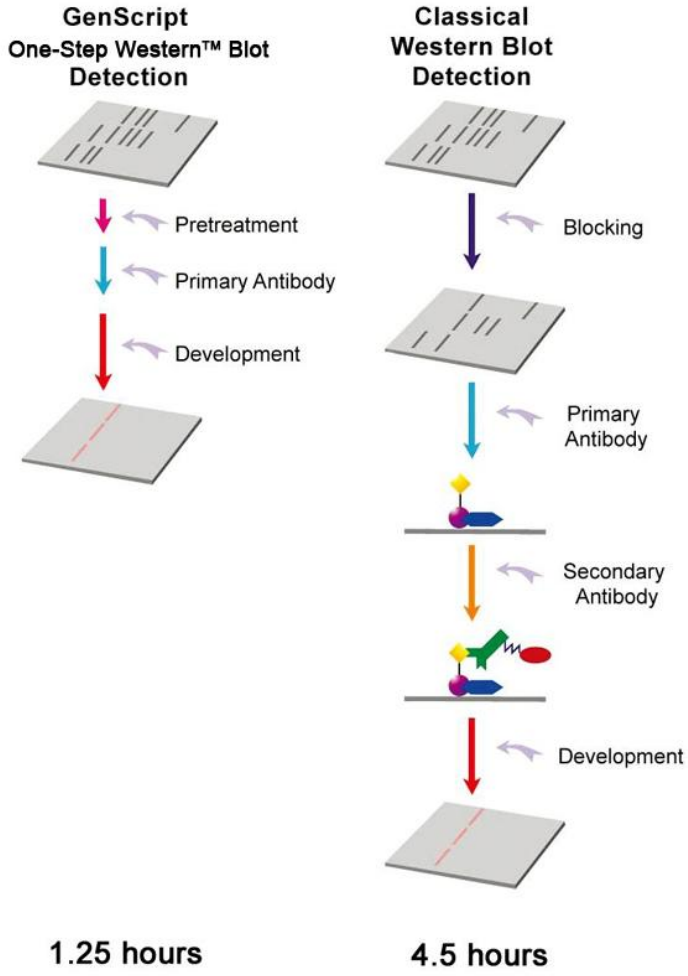

Figure 1. Overview of Western Procedures

II. KIT CONTENTS

Each kit contains enough reagents for 10 mini gel (7.5 x 8 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

The One-Step Western™ Trx Detection Kit has applications that include the following:

- Detection of Trx or Trx-tagged proteins
- Confirmation of Trx-tagged protein expression
- Screening of Trx-tagged protein expression for optimization

IV. KEY FEATURES

- Easy to perform: Fewer steps mean fewer chances for human error.
- High sensitivity and low background: It contains WestClear™ Nitrocellulose Membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit, optimized for low background.
- Reproducible results: The kit produces highly reproducible results.
- The kit exhibits excellent linearity.
- The One-Step Western™ Kit needs far less optimization than the classical three-step method.
- Neither a primary antibody nor a secondary antibody is needed.

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 of its components**.

VI. ONE-STEP WESTERN™ 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.

Before use, prepare the following:

1. Gently invert each solution bottle several times to mix well.
2. 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.
3. 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.

Western or Dot blot procedure:

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 14 ml of 1X wash solution two times.
2. Incubate the membrane from step 1 with the WB solution for 40 minutes on a shaker 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. Mix 3 ml of LumiSensor™ reagent A with 3 ml of LumiSensor™ reagent B to make the working solution (0.1 ml/cm^2). Drain off the excess wash solution from the membrane by holding the membrane vertically with forceps and touching its edge against a tissue. Place the membrane on a clean, flat surface, and cover it with working solution.
5. 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.
6. 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 Trx protein

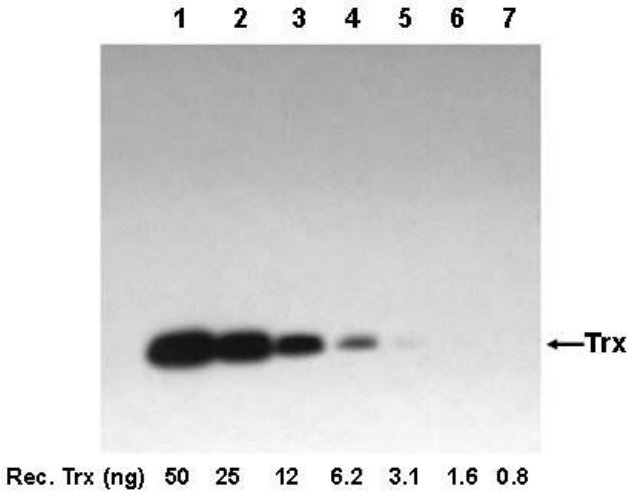

Figure 2. Western blot for the detection of recombinant Trx protein using One-Step Western™ Trx Detection Kit L00213. 50 ng, 25 ng, 12 ng, 6.2 ng, 3.1 ng, 1.6 ng, and 0.8 ng of Trx were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 6 and Lane 7, respectively. The blot was developed with the LumiSensor™ system 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 is loaded.	Load more protein(s) onto the SDS-PAGE gel.
	There is poor transfer efficiency.	Optimize transfer time and/or electrical current. Make sure that there are no air bubbles between the membrane and gel.
	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, longer incubation time may be needed.
There is high background and/or non-specific bands on the blot.	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 reagents or equipment is contaminated.	Use a clean container every time you change solution for the rinse and wash steps. Wear gloves and use clean forceps to handle membranes.
	There is excess working solution.	Remove excess working solution using a soft clean tissue.

IX. ORDERING INFORMATION

One-Step Western™ Trx Detection Kit: Cat. No. L00213

Patent Pending.

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