One-Step Western™ GST Detection Kit

Technical Manual No. 0200 **Version 03262008**

I. DESCRIPTION

The One-Step Western™ GST Detection Kit is designed for fast and reliable detection of GST and GST-fusion proteins. Based on GenScript's revolutionary One-Step Western™ Detection technology, this kit sidesteps the cumbersome classical three-step procedure of blocking, primary antibody binding, and secondary antibody binding and replaces it with a simple, one-hour procedure that yields highly reproducible results each time.

First, transfer the proteins from your gel to the provided Western membrane. Then incubate the membrane in our pretreat solution mixture for five minutes and follow with incubation in WB solution for 40 minutes. Then wash the membrane three times for five minutes each. The blots are now ready for development. At right, we have compared the GenScript One-Step Western™ blot procedure to the classical three-step procedure in figure 1.

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

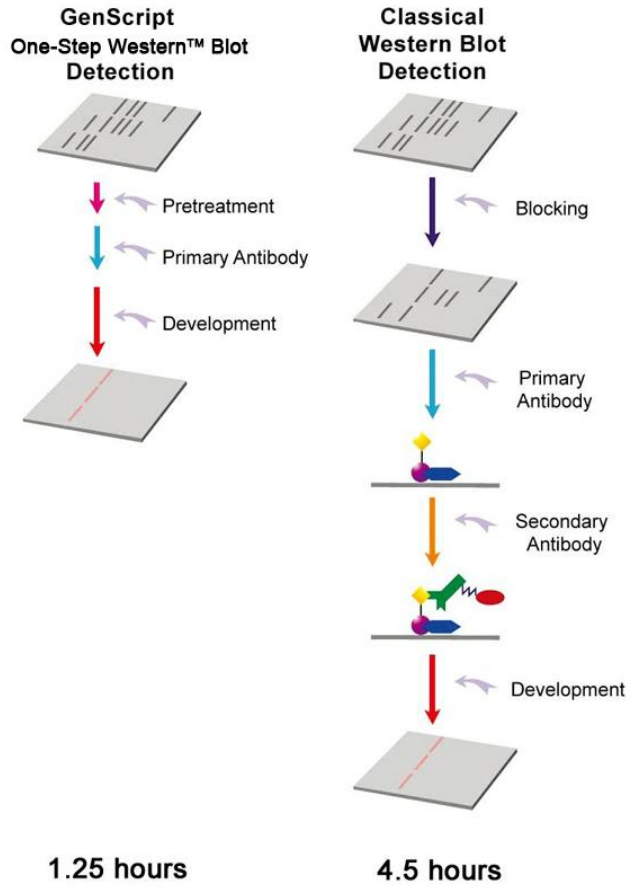

Figure 1. Overview of Western Procedure

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™ GST Detection Kit enables Western- or Dot-bloting for numerous applications:

- Detecting GST or GST-fusion proteins
- Checking GST or GST-fusion protein expression
- Screening GST-fusion protein expression for optimization

IV. KEY FEATURES

- Easy to perform: Our simple and rapid procedure prepares a complete Western blot or Dot blot for development in about one hour.
- High sensitivity and low background: Sensitivity is comparable with or better than that of the classical 4.5-hour procedure.
- Reproducible results.
- Excellent linearity.
- No optimization is needed.
- Neither a primary antibody nor a secondary antibody is needed.

V. STORAGE

Store the 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™ GST KIT PROTOCOL

Transfer the proteins from gel to membrane before running the kit. The following 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 membrane.

Before use, do 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 blot 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 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 every time you change solutions for the rinse and wash steps 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 clean, flat surface, and cover it with the 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 the membrane in a clean piece of plastic film.
6. Expose the membrane to a sheet of film for 30 seconds and then develop the film. Repeat this step with different exposure times for best results.

VII. EXAMPLES

Dot blot detection of GST protein:

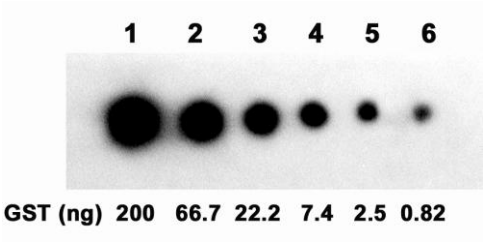

Figure 2. The Dot blot detection of GST protein using One-Step Western™ GST Detection Kit (GenScript, L00211). 200 ng, 66.7 ng, 22.2 ng, 7.4 ng, 2.5 ng and 0.82 ng of GST were spotted on the membrane. The blot was developed with the LumiSensor™ system included in the kit.

Western blot detection of GST protein:

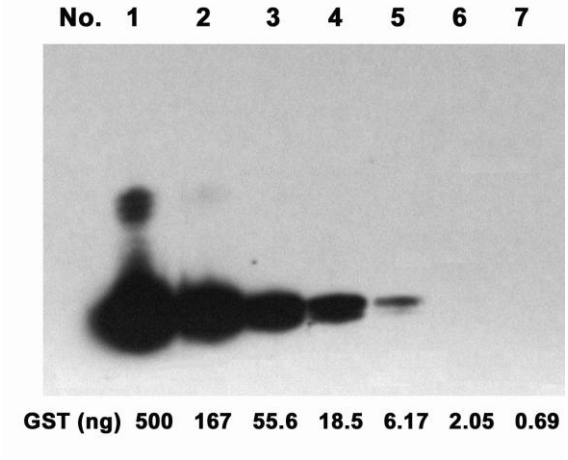

Figure 3. The Western blot detection of GST protein using One-Step Western™ GST Detection Kit (GenScript, L00211). 500 ng, 167 ng, 55.6 ng, 18.5 ng, 6.17 ng, 2.05 ng and 0.69 ng of GST were loaded in Lane 1, Lane 2, Lane 3, Lane 4, and Lane 5, respectively. The blot was developed with the LumiSensor™ 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 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 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 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. Wear gloves and use clean forceps to handle the membranes.
	There is excess working solution.	Remove excess working solution using a soft clean tissue.

IX. ORDERING INFORMATION

One-Step Western™ GST Detection Kit: Cat. No.L00211

Patent Pending.

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GenScript Corporation
120 Centennial Ave., Piscataway, NJ 08854
Tel: 732-885-9188, 732-885-9688
Fax: 732-210-0262, 732-885-5878
Email: info@genscript.com
Web: http://www.genscript.com