

One-Step Western™ Kit using TMB



Technical Manual No. 0203

Version 03272008

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I. DESCRIPTION

The One-Step Western™ Kit with TMB contains all the necessary reagents, buffers, nitrocellulose membrane and TMB substrate for performing a 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 using ChromoSensor™ One Solution TMB substrate included in the kit. The One-Step Western™ procedure is contrasted with a classical western at right.

The kit contains 0.2 µm WestClear™ nitrocellulose membrane and ChromoSensor™ One-Solution TMB substrate optimized for best results. WestClear™ nitrocellulose membrane and ChromoSensor™ One Solution TMB substrate are also available separately.

Currently, GenScript provides two different One-Step Western™ Kits with TMB, one for rabbit primary antibodies and one for mouse primary antibodies. Both kits are effective for both Western and Dot blots.

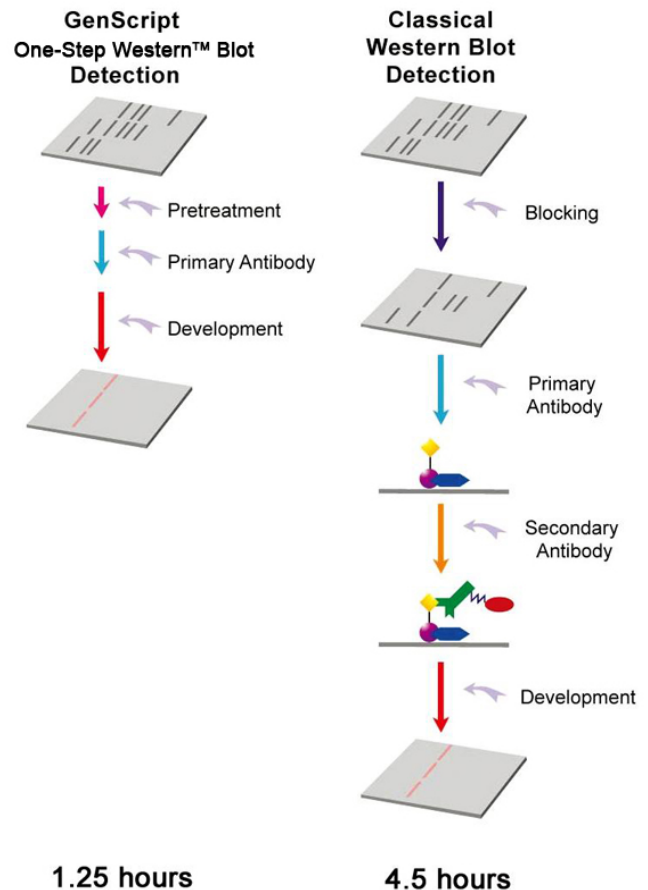


Figure 1. Overview of Western Procedure



II. KIT CONTENTS

There are two different One-Step™ Western Kits available with TMB, kit L00204T for rabbit primary antibodies (whole molecule), and kit L00205T for using primary antibodies. Each kit contains enough reagents for 10 mini gel (7.5 x 8 cm) Western or Dot blots.

Kit Components L00204T, L00205T	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
One solution TMB	60 mL
Protocol	1

Product	Catalog No.
Kit For Rabbit Primary Antibody	L00204T
Kit For Mouse Primary Antibody	L00205T

III. APPLICATIONS

The One-Step Western™ Kit with TMB has applications that include the following:

- Protein (antigen) detection
- Confirmation of protein expression
- Antibody titration
- Antigen titration

IV. KEY FEATURES

- ◆ Easy to perform: Fewer steps mean fewer chances for human error.
- ◆ Requires no darkroom or film.
- ◆ Low background: The kit also contains ChromoSensor™ One-Solution TMB substrate, optimized for minimal background.
- ◆ High sensitivity: The sensitivity of this one-hour kit is comparable to or better than that of the classical 4.5-hour procedure, depending on the quality and quantity of antibody used.
- ◆ Reproducible results: The kit produces highly reproducible results.
- ◆ Needs far less optimization than the three-step method.
- ◆ Needs no secondary antibody.

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 cm² membrane. The volumes of the reagents can be scaled up or down according to the size of the membrane.

Reagents not provided:

Primary antibodies. Purified monoclonal or affinity-purified polyclonal antibodies are preferred. Rabbit polyclonal antibodies should be whole-molecule, Fab fraction gives a significantly lower signal.

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 ddH₂O 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:

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 (mixture of pretreat A and pretreat B) 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 on a shaker with the WB solution containing the appropriate primary antibody for 40 minutes at room temperature.
3. Rinse the membrane once with 1X wash solution. Then wash the membrane on a shaker two times for five minutes each with 1X wash solution. Use a clean container for each rinse and wash step to avoid carryover contamination and to reduce background.
4. Develop the blot with 6 ml of ChromoSensor™ One-Solution TMB substrate (0.1 ml/cm²) until the desired color is reached. Stop the reaction by rinsing the membrane three times for 30 seconds each in 20 ml of deionized water.



VII. EXAMPLES

Western blot detection of housekeeping protein α -Tubulin

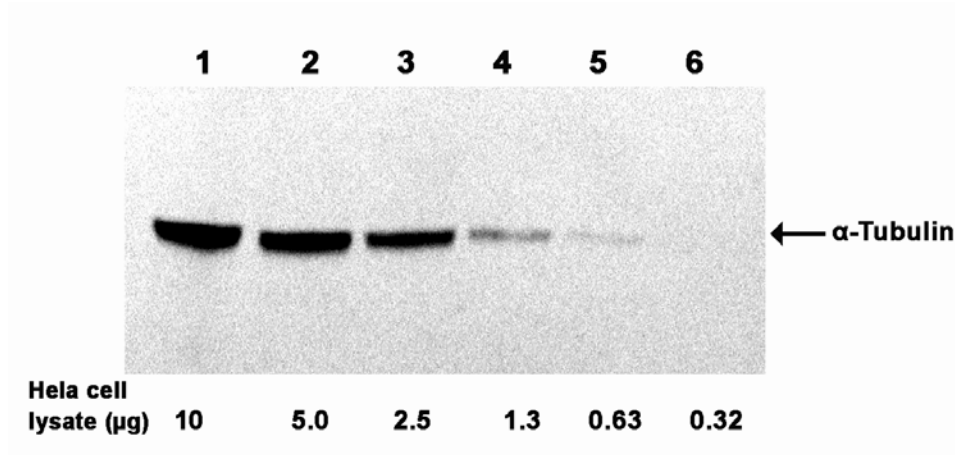


Figure 2. Western blot detection of α -Tubulin using One-Step Western™ Kit Complete Using TMB (GenScript, L00205T) and α -Tubulin Monoclonal Antibody (abcam, ab7291). 10 μ g, 5.0 μ g, 2.5 μ g, 1.3 μ g, 0.63 μ g, and 0.32 μ g of *Hela* cell lysate (BD Biosciences, 611449) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, Lane 5, and Lane 6, respectively. The blot was developed with the ChromoSensor™ One-Solution TMB 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 transfer time and/or electrical current. Make sure that there are no air bubbles between the membrane and the 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 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.	There is non-specific binding/cross-reactivity of primary antibody.	Select highly specific primary antibody. Purified monoclonal or affinity-purified polyclonal primary antibodies are preferred.
	There is too much primary antibody in the One-Step Western™ Blot Solution.	Reduce the concentration of the primary antibody added to the WB solution. Optimize the antibody concentration using a Dot blot.
	The wash time is too short	Adding a third wash with 1X wash solution after primary antibody binding 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 membranes.



IX. ORDERING INFORMATION

One-Step Western™ Kit using TMB: L00204T for use with rabbit primary antibodies.
L00205T for use with mouse primary antibodies.

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Patent Pending.

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