

One-Step Western™ His-Tag Kit

Technical Manual No. 0207

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I. DESCRIPTION

The One-Step Western™ His-Tag Kit yields a journal-quality western blot in about 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 for 40 minutes, and lastly, wash three times for five minutes each. The membrane is then ready for development. The One-Step Western™ procedure is contrasted with a classical western at right.

This kit can detect (His)₄ or (His)₅ or (His)₆ tags fused to any part of the relevant protein, the N-terminus, C-terminus, or anywhere in between. Occasionally, protein folding may prevent access to and detection of the His-tag. Treatment of the membrane with 3 M thiocyanate¹ may expose the His-tag.

The One-Step Western™ His-Tag Kit contains all the necessary reagents, buffers, nitrocellulose membrane and HRP substrate for performing Western or Dot blot. Neither primary nor a secondary antibody is needed. A sensitive chemiluminescent substrate is also included for HRP signal development.

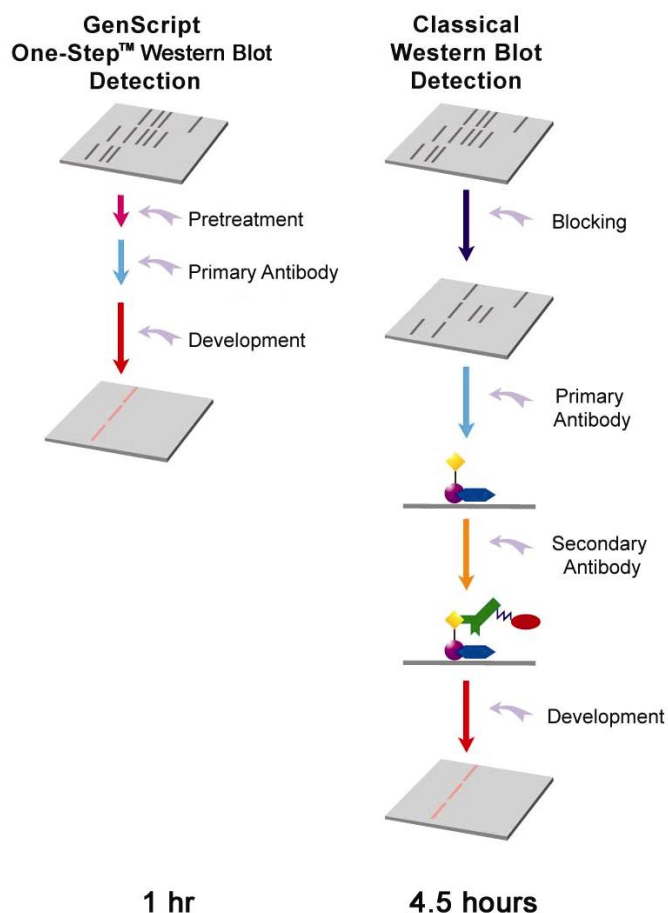


Figure 1. Overview of Western Procedure.



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

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

- Detection of His-tagged proteins
- Confirmation of His-tagged protein expression
- Screening of His-tagged protein expression for optimization

IV. KEY FEATURES

- ◆ Easy to perform: Fewer steps mean fewer chances for human error.
- ◆ High sensitivity and low background: The kit is optimized to give strong signal and low background.
- ◆ Reproducible results: The kit produces highly reproducible results.
- ◆ Excellent linearity.
- ◆ Needs no optimization.
- ◆ 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™ HIS-TAG 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. Use this kit after transferring proteins from gel to membrane.

Before use, prepare the following:

1. Gently invert each solution bottle several times to mix well.
2. Dilute 12.5 ml of 10X wash solution with 112.5 ml of distilled or filtered water to make a 1X wash solution, use 20 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 just before use in a plastic container such as Western Wash Box (GenScript, M00100) to make the pretreat solution mixture.

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 (fresh 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 20 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 20 ml of 1X wash solution. Then wash the membrane on a shaker three times for five minutes each with 20 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/cm²). 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 the membrane 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 to a sheet of film for 30 seconds and develop the film. Repeat this step with different exposure times to get the best results.

VII. EXAMPLES

Western blot detection of His-tagged protein markers

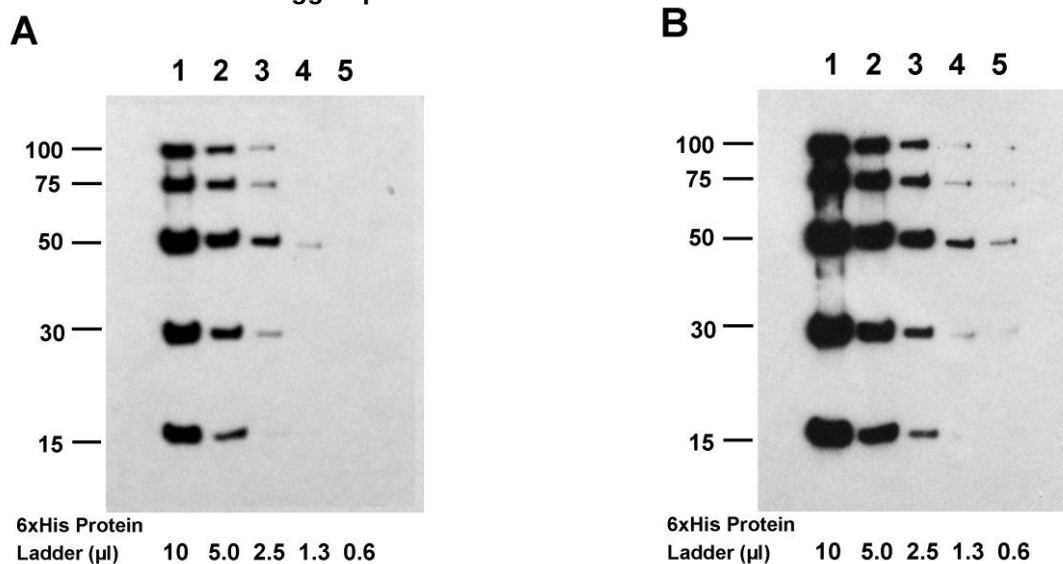


Figure 2. Western blot detection of 6xHis Protein Ladder (Qiagen, Cat. No. 34705) using the One-Step Western™ His-Tag Detection Kit L00212. 10 µl, 5.0 µl, 2.5 µl, 1.3 µl, and 0.6 µl of the 6xHis Protein Ladder (reconstituted in 250 µl 1X SDS-PAGE sample buffer as suggested by Qiagen) were loaded in Lane 1, Lane 2, Lane 3, Lane 4, and Lane 5, respectively. The blot was developed with LumiSensor™ system included in the kit. Picture A above was given an exposure time of 30 seconds and picture B 60 seconds.

In Lane 5, where 0.6 µl of the 6xHis Protein Ladder was loaded, 12.5 ng of 50 kDa His-tagged protein was cleanly detected.



VIII. TROUBLESHOOTING

Problem	Probable Cause	Solution
The signal is weak or invisible.	There is too little protein 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.	In most cases, a 40-minute incubation at room temperature is enough. However, a longer incubation time may be needed to detect low amount of antigens.
There is high background and/or non-specific bands on the blot.	The wash time is too short.	Adding an additional wash step after WB binding step 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 every time you change solution for the rinse and wash steps. 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™ His-Tag Kit: Cat. No. L00212

X. REFERENCES

1. Jin, L., Wei, X., *et al.* (1995). Use of alpha-N,N-bis[carboxymethyl]lysine-modified peroxidase in immunoassays. *Anal. Biochem.* 229:54-60.

Patent Pending. For Research Use Only.

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