One-Step Western™ DYKDDDDK-Tag Kit

Technical Manual No. 0212 **Version 03282008**

I. DESCRIPTION

The One-Step Western™ DYKDDDDK-Tag Kit yields a journal-quality Western or Dot blot detection of DYKDDDDK-tagged proteins 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 for 40 minutes, and lastly, wash three times for ten minutes each. The membrane can then 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 DYKDDDDK-tags fused to any part of the protein, the N-terminus, C-terminus or interior.

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

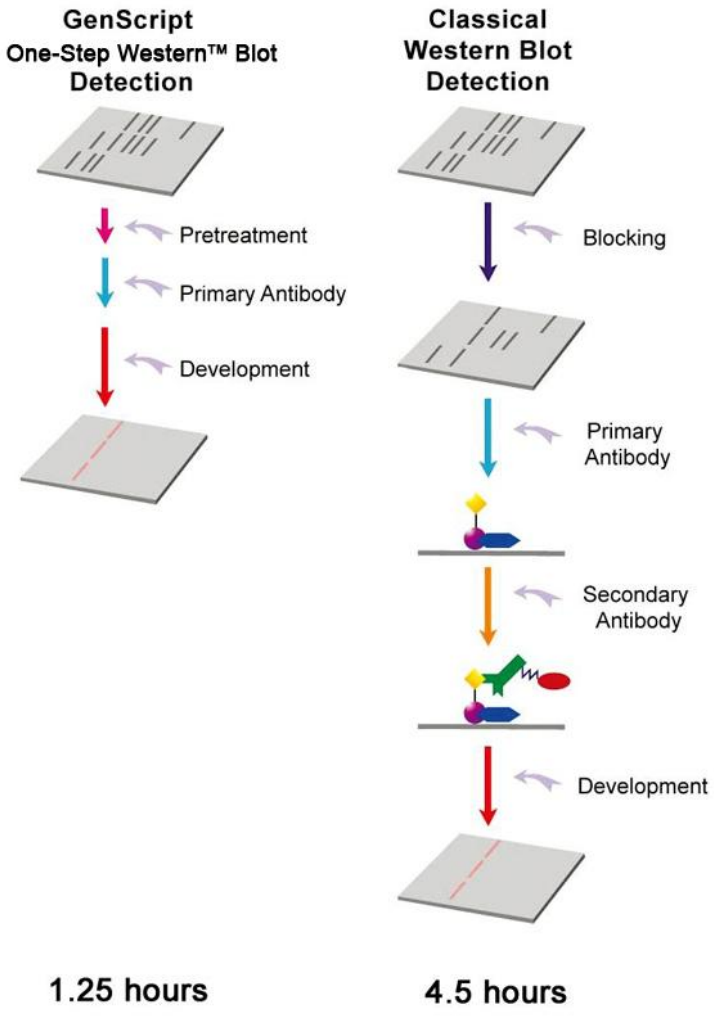

Figure 1. Overview of Western Procedure.

II. KIT CONTENTS

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

Kit Component	10 Assays
Pretreat A solution	100 ml
Pretreat B solution	100 ml
WB solution	100 ml
10X Wash solution	125 ml
WestClear™ Nitrocellulose Membrane (0.2 µm, 7.5 x 8 cm)	10 Sheets
LumiSensor™ Plus Chemiluminescent HRP Substrate	2 x 30 ml
Protocol	1

III. APPLICATIONS

One-Step Western™ DYKDDDDK-Tag Kit has applications that include the following:

- Detection of DYKDDDDK-tagged proteins.
- Confirmation of DYKDDDDK-tagged protein expression.
- Screening of DYKDDDDK-tagged protein expression for optimization.

IV. KEY FEATURES

- Easy to perform: Fewer steps mean fewer chances for human error.
- Low background: The kit contains WestClear™ Nitrocellulose Membrane and LumiSensor™ Chemiluminescent HRP Substrate Kit, optimized for low background.
- High sensitivity: The kit's sensitivity is comparable with or better than that of the classical 4.5-hour procedure, depending on the quality and amount of antibodies used.
- Reproducible results: The kit produces highly reproducible results.
- No antibody is needed.
- The One-Step Western™ Kit needs far less optimization than the classical three-step method.

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™ DYKDDDDK-Tag PROTOCOL

This procedure is optimized for a sheet of 7.5 x 8 cm membrane, the volumes of reagents can be scaled up or down according to the size of 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 ddH_2O 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 on. 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™ Plus reagent A with 3 ml of LumiSensor™ Plus 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 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 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 DYKDDDDK-tagged protein expressed in *E. coli* cells:

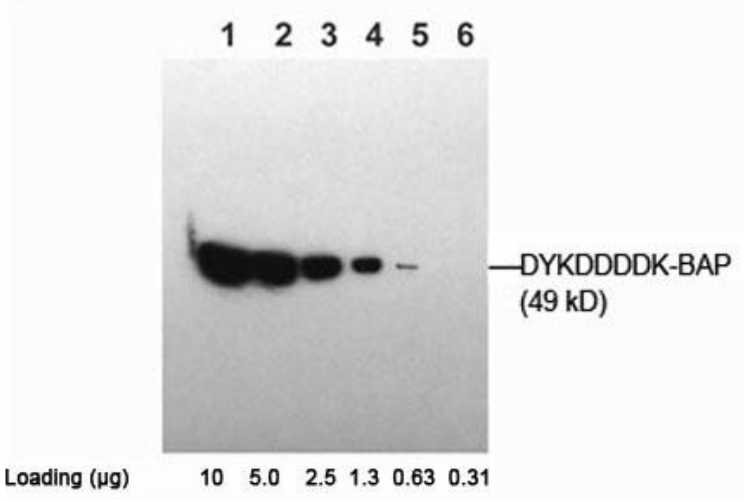

Figure 2. Western blot detection of DYKDDDDK-tagged protein using One-Step Western™ DYKDDDDK-Tag Kit (GenScript, L00215). 10 µg, 5.0 µg, 2.5 µg, 1.3 µg, 0.63 µg, and 0.31 µg of DYKDDDDK-BAP (Sigma, P7582) were loaded in lane 1-6, respectively. The blot was developed with LumiSensor™ system included in the kit. Exposure time was two minutes.

Western blot detection of DYKDDDDK-tagged protein expressed in mammalian cells:

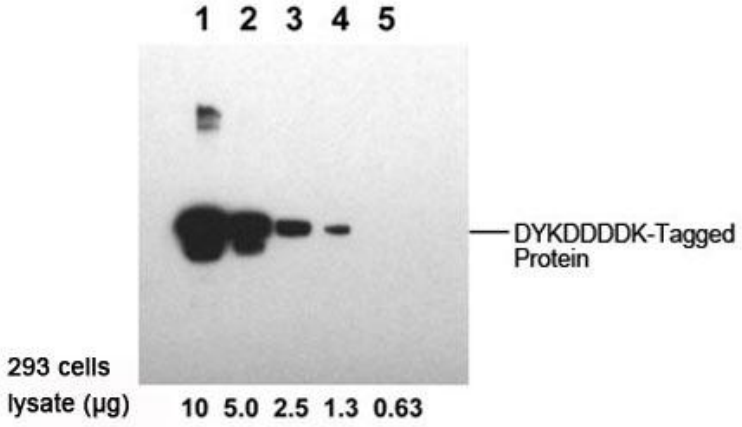

Figure 3. Western blot for the detection of DYKDDDDK-tagged protein using One-Step Western™ DYKDDDDK-Tag Kit L00215. 10 µg, 5.0 µg, 2.5 µg, 1.3 µg, and 0.63 µg of DYKDDDDK lysate (GenScript, M0005. 293 cells transfected with DYKDDDDK-tagged minigene, 60 kDa) were loaded in lane 1-5, respectively. The blot was developed with LumiSensor™ system included in the kit. Exposure time was two minutes.

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 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, a longer incubation time may be needed to detect low amount of antigens.
There is high background or non-specific bands on the blot.	The wash time is too short.	Adding one more wash with 1X wash solution after WB binding step always decreases 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 rinse and wash. 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™ DYKDDDDK-Tag Kit: Cat. No. L00215

Patent Pending. For Research Use Only.

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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