ONE-HOUR Western™ General Guideline Using SNAP I.D.

Technical Manual No. TM0357 **Version 06242009**

I. Description

Under ordinary conditions one can perform a complete Western blot in just one hour or so using GenScript's ONE-HOUR Western™Blot Kits. When used in combination with the Millipore SNAP I.D. System, one can perform a complete Western blot even faster, in less than 12 minutes.

Procedure	Time
Blocking	20 sec
Antibody Incubation	10 min
Wash	1 min
Total	**< 12 min**

II. Protocol

1. Prepare Mixture 1

Before or during protein electrophoresis, prepare Mixture 1 by mixing primary antibody with WB-1 in a microcentrifuge tube as indicated below. Vortex Mixture 1 gently for a few seconds and centrifuge briefly to collect the solution at the bottom of the tube. Incubate Mixture 1 at room temperature (RT) for at least 40 minutes.

Mixture 1 Preparation	Single-Well	Double-Well	Triple-Well
WB-1 Solution	30 µl per well	15 ul per well	10 µl per well
Primary Antibody*	3 µg per well	1.5 µg per well	1 µg per well

***Refer to manufacturer's recommendations for the appropriate amount of antibody. GenScript's ONE-HOUR Advanced Western™ Kits (L00241, L00242, and L00243) use only 1/4 of the manufacturer's recommended amount of the primary antibody for Western blot.

2. Pre-Treat Membrane

Just before the protein transfer from gel to membrane is complete, prepare the Pretreat Solution:

Mix one volume of Pretreat A, one volume of Pretreat B, and two volumes of double distilled water. Use fresh mixture for every analysis. Use the Pretreat Solution as indicated below. Apply vacuum until well(s) are completely empty.

Pretreat Solution Preparation	Single-Well	Double-Well	Triple-Well
Pretreat A	5 ml per well	2.5 ml per well	1.25 ml per well
Pretreat B	5 ml per well	2.5 ml per well	1.25 ml per well
ddH_2O	10 ml per well	5 ml per well	2.5 ml per well

Wash with 1X Wash solution once, using 20 ml for Single-Well, 10 ml for Double-Well, etc.

3. Antibody Incubation with Pre-Treated Membrane

Mix the Mixture 1 solution prepared in step 1 with WB-2 Solution in the amounts indicated below. Incubate the membrane in this solution for 10 minutes at room temperature with the vacuum turned off.

Antibody Incubation	Single-Well	Double-Well	Triple-Well
WB-2 solution	3 ml per well	1.5 ml per well	1 ml per well

4. Wash

With the vacuum running continuously, wash three times with 1X Wash Solution.

Wash	Single-Well	Double-Well	Triple-Well
1X Wash solution	3 X 20 ml	3 X 15 ml	3 X 10 ml

5. Signal Development

Remove the membrane from the SNAP I.D system and incubate it with a detection reagent such as LumiSensor™ HRP substrate, LumiSensor™ plus HRP substrate, or TMB substrate. When using GenScript fluorescence kits (L00397 and L00398), the membrane must be scanned with an appropriate fluorescence imager, such as LI-COR Odyssey Infrared Imaging Systems.

For Research Use Only.

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: www.genscript.com