Allied Biotech

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Discovery Products

User Manual
One-hour One-step™ Western Blot Kits

(For R&D Use Only)

Product Name, Catalog Number & Content:

Product Name	Cat #	Size	Content
One-hour-One-step TM Western Blot Kit-R With SuperHi ECL Substrate (Suitable for Rabbit Primary Antibodies)	WB-R51 ($245)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-R51 (2 ML) 10X Rapid Wash Solution (100 ML) SuperHi™ Chemiluminescence (40ML) User Manual (One copy).
One-hour-One-step TM Western Blot Kit-M With SuperHi ECL Substrate (Suitable for Mouse Primary Antibodies)	WB-M51 ($245)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-M51 (2 ML) 10X Rapid Wash Solution (100 ML) SuperHi™ Chemiluminescence (40ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-G With SuperHi ECL Substrate (Suitable for Goat Primary Antibodies)	WB-G51 ($245)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-G51 (2 ML) 10X Rapid Wash Solution (100 ML) SuperHi™ Chemiluminescence (40ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-R With ECL Substrate (Suitable for Rabbit Primary Antibodies)	WB-R52 ($225)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-R52 (2 ML) 10X Rapid Wash Solution (100 ML) Chemiluminescence (40ML) User Manual (One copy).
One-hour-One-step TM Western Blot Kit-M With ECL Substrate (Suitable for Mouse Primary Antibodies)	WB-M52 ($225)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-M52 (2 ML) 10X Rapid Wash Solution (100 ML) Chemiluminescence (40ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-G With ECL Substrate (Suitable for Goat Primary Antibodies)	WB-G52 ($225)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-G52 (2 ML) 10X Rapid Wash Solution (100 ML) Chemiluminescence (40ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-R With DAB Substrate (Suitable for Rabbit Primary Antibodies)	WB-R50 ($190)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-R50 (2 ML) 10X Rapid Wash Solution (100 ML) 100X DAB Solution Set (2 ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-M With DAB Substrate (Suitable for Mouse Primary Antibodies)	WB-M50 ($190)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-M50 (2 ML) 10X Rapid Wash Solution (100 ML) 100X DAB Solution Set (2 ML) User Manual (One copy)
One-hour-One-step TM Western Blot Kit-G With DAB Substrate (Suitable for Goat Primary Antibodies)	WB-G50 ($190)	Kit (10 tests)	Enhancer (100 ML) 50XWB Probe-G50 (2 ML) 10X Rapid Wash Solution (100 ML) 100X DAB Solution Set (2 ML) User Manual (One copy)

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Product Description:

One-hour-One-step™ Western Blot Kit (patent pending) is an innovative and the most advanced Western blot kit. By using the kit, your Western blot will be no longer a time-consuming and labor-intense procedure. The procedure of a typical (regular) Western blot contains seven steps (blocking, washing, primary antibody-binding, washing, secondary antibody-binding, washing, and developing) and requires 4~5 hours. In contrast, the procedure of One-hour-One-step WB only contains: one-step reacting, washing, and developing, and requires as short as 30 minutes. Currently, we provide twelve types of One-hour-One-step WB kit as shown on the above table. Each kit is sufficient for detecting 10 (without reuse) to 40 (if reused) mini gel-size membranes.

Application (for R&D use only):

Rapid Immuno-blot (Western-blot, Dot-blot) for:

- Rapidly identifying a specific protein either purified or unpurified.
- Rapidly detecting a specific protein expressed in cells.
- Rapidly monitoring a target protein in a purification procedure (just using a small amount of samples, 2μl).
- Rapidly semi-quantifying a specific protein using just 2μ of sample.
- Rapidly screening or titrating antigen-specific antibodies.

Features:

1 Easy: a simple one-step reaction (a regular procedure needs 4-step: blocking, 1st antibody-binding, washing and 2nd antibody-binding).
2 Rapid: the whole procedure takes less than one hour (a regular immuno-blot takes 3~5 hrs).
3 Universal: suitable for most primary antibodies.
4 Sensitive: comparable sensitivity with regular immuno-blot.
5 Reusable: antibodies and reagents can be reused.
6 Restainable: membrane can be re-probed with different antibodies.
7 Reproducible: result is highly reproducible.

Storage:

	Enhancer	WB Probe	10X Rapid Wash Solution	100X DAB solution set	Chemiluminescence (SuperHi or Non-SuperHi)	Stripping Buffer
store at -20 °C	1 year	6 months	> 1 year	6 months	6 months	> 1 year
store at 2~8 °C	6 months	4 month	> 1 year		6 months	> 1 year
store at room temperature			1 year			1 year

Note: Kit will be shipped in ambient temperature.

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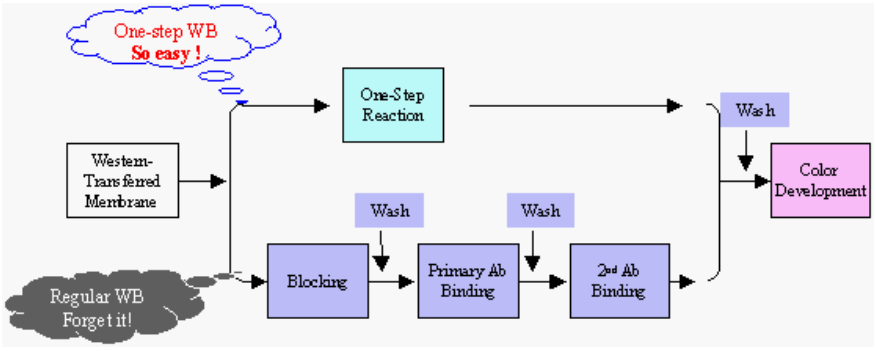
Comparison of One-hour-One-step WB with Regular WB:



Protocol:

I. Additionally required materials (not supplied in the kit):

Primary antibody: Purified monoclonal or affinity-purified polyclonal antibodies (antigen-specific IgGs) are preferred. Although unpurified antibody or serum is also suitable for the One-step WB, user may need to optimize both concentrations of antibody and antigen to avoid producing a poor ratio of signal to noise.

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Note: We currently provide different types of One-hour-One-step WB kits. Kits labeled with -R are suitable for using together with rabbit primary antibodies, with -M suitable for using together with mouse primary antibodies, and with -G suitable for using together with goat primary antibodies.

Western-blot Membrane: Normal Western-blot membranes (such as Nitrocellulose or PVDF membrane) are suitable for One-step WB. User must perform PAGE and Western-transferring ahead of using this protocol.

X-ray film & its related reagents: Required only if Chemiluminescent substrate is used. Sensitive X-ray films are preferred. X-ray film developing related reagents (excluding Chemiluminescence) and apparatus (such as film cassettes and developer trays or auto-film-developing machine) are also required.

II. Procedure:

Flowchart: One-hour-One-step Reaction (30-50 min) --- **> Wash** (3-5min) ---**> Development** (1-5min).

1. **One-hour-One-step Reaction**

- Soak both sides of Western-transferred or dot-blotted membrane with 10ml of WB Enhancer (supplied in the kit) and gently shake at room temperature for 5 minutes (if Chemiluminescence for consequent development is used), or 0-3 minutes (if DAB for consequent development is used).
- Mix 5ug of primary antibody and 200ul of WB Probe (supplied in the kit), or with the recommended amount in the following table, add this Antibody-Probe Mixture to the above 10-ml Enhancer and continue shaking for 30-50 minutes at room temperature.

Reagent	Amounts of Antibody and Probe to be mixed with 10 ml WB- Enhance			
	For DAB-development.		For Chemiluminescence-development	
Primary Ab (normal)	10~20 ug		3~10 ug	
Primary Ab (high affinity)		3~10 ug		< 3 ug
Probe	200 ul	200 ul	100-200 ul	100-200 ul

Tips: 1. The addition of the amount of primary antibody is highly depending on its purity and antigen-specific affinity. If you don't know your primary Ab is high affinitive or not, there are two ways to go: 1). Treat your primary Ab as a normal one and do your test. If the read-out signal accompanies a high background, wash the membrane once with Stripping Buffer (see step 4 below) then restain it with a lower concentrations of Primary Ab (or both primary Ab and Probe); or 2). Treat your primary Ab as high affinitive and do your test. If the read-out signal is too weak, wash the membrane with distilled water then place it back to the original reaction mixture and restain it after adding additional amount of primary Ab (or both primary Ab plus Probe). **2.** The membrane must be fully covered by solution during shaking. **3.** If using WB Enhancer taken at refrigerator temperature, extend the incubation time for an additional 15 min. **4.** For convenience, the one-step reaction can be left overnight at 4 °C.

- If desired, recover this used One-step Reaction Mixture (consisting of Enhancer, primary antibody and WB Probe) and refrigerate at 2-8 °C. The One-step Reaction Mixture can be subsequently reused 3-4 times for testing the same antigen.

2. **Wash:**
- Prepare 1X Rapid Wash Solution: Shake the bottle of 10X Rapid Wash Solution (as it might contain precipitate) for 10 sec, then dilute the solution with 9 times volume of distilled water.
- Briefly rinse the membrane (from step 1) with plenty of distilled water to remove free antibody and WB Probe.
- Wash the membrane for 2 minutes with 30 ml of 1X Rapid Wash Solution by aggressively shaking (to remove non-specific binding substances); Rinse the membrane again with plenty of distilled water.
- Repeat the above wash and rinse for another 1 to 2 times (repeat at least twice if SuperHi chemiluminescence is used for development).

3. **Development**:
Using normal Chemiluminescence or SuperHi Chemiluminescence supplied in kits WB-R52, WB-M52 and WB-G52 or WB-R51, WB-M51 and WB-G51:

- Place the water-rinsed membrane (from step 2) in an X-ray film cassette (protein side up).
- Mix 2 ml Solution A and 2 ml Solution B of the chemiluminescent substrate, and drop 0.5 ml of this developing solution onto the membrane to cover the whole protein side.
- Cover the membrane from one end to the other with a transparent film or clear Sara Wrap. When covering the membrane press the cover sheet in order to remove bubbles and excessive developing solution between cover sheet and membrane.
- Transfer the cassette to a dark room and expose an X-ray film on the covered membrane (protein side facing the X-ray film) for a suitable time period, usually 10 seconds to 5 minutes. (It is recommended to expose multiple X-ray films for different time periods).
- Develop the X-ray film in darkness or use an automatic developing machine.

Tips: SuperHi Chemiluminescence is a very sensitive substrate. If developed X-ray films are too dark, perform one or combination of the following actions: 1) simply reduce the exposure time to as short as 1 second; 2) extensively wash the membrane for another 5 min; 3) dilute the mixed A & B developing solution 2-3 times with distilled water; 4) re-probe the membrane with a lower concentration of WB Probe & Primary Antibody after washing with Stripping Buffer (see step 4 below).

Using DAB substrate (supplied in kits WB-R50, WB-M50 and WB-G50):

- Take 100µl of the 100X DAB solution and 100µl of 100X D-buffer, and mix them with 10ml of distilled water.
- Incubate this DAB mixture with the membrane (from step 2) at room temperature for a suitable time period, usualiy 3-10 minutes, till desired color blots appear.
- Wash the membrane with plenty of distilled water then air-dry it.

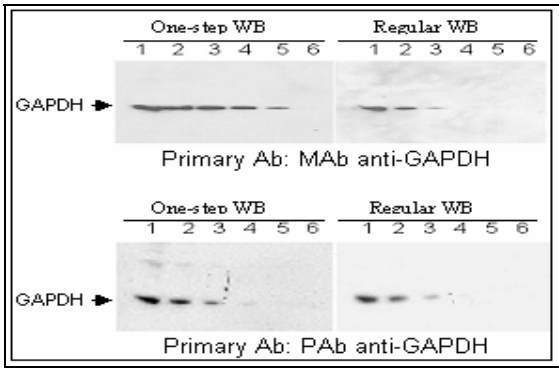
4. **(Optional) Blotting with different antibody:** If needed, the membrane after development can be reused for further staining with different primary antibody. This procedure will effectively use your valuable Western-transferred membrane.

   - Shake the membrane (from step 3) with 10 ml of Stripping Buffer (Cat # 238-51) for 5 min, wash it one time with 10ml of PBS buffer and then rinse it with plenty of distilled water. Recover the Stripping Buffer for reusing in future.
   - Repeat the above steps 1 to 3, but use different primary antibody at step 1.

Application Examples:

I. Detection of unpurified proteins in cell lysate.

One-step WB is the fastest and the simplest Western blot in detection of specific proteins expressed in cells.



Comparison of One-step WB (1 hr) to Regular WB (4 hrs) in detection of GAPDH proteins in human cell lysate.

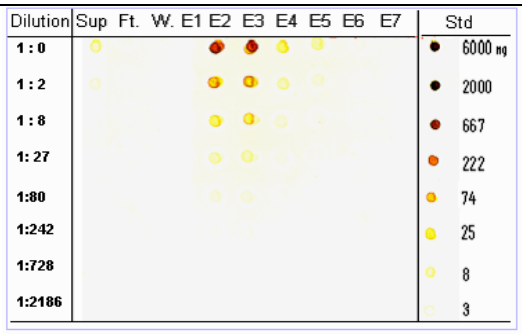
Upper left: One-step WB using rabbit anti-GAPDH polyclonal antibodies.
Upper right: Regular WB using rabbit anti-GAPDH polyclonal antibodies.
Lower left: One-step WB using mouse anti-GAPDH monoclonal antibody.
Lower right: Regular WB using mouse anti-GAPDH monoclonal antibody.

Loaded cell numbers (lysate): from lane 1 to lane 6 are 10K, 3K, 1K, 0.3K, 0.1K and 0.01K respectively.

Blots were developed with Chemiluminescence.

II. Rapid monitoring and semi-quantifying target protein existing in purification fractions.

One-step WB provides a very quick and simple way to monitor a target protein during purification. Not only is it a sensitive method (can detect <10 ng/dot by DAB or <0.1 ng/dot by Chemiluminescence), but also a method that requires sample amount as small as 1-2ul per dot test. Moreover, it can be used as a quick semi-quantitative assay if a standard with known protein concentration is employed.



30-minutes semi-quantifying target protein (P50-flag) existing in purification fractions. Serially diluted samples (left side) and standard (right side) were dot-blotted onto an NC membrane (1µl/dot) and then detected with mouse anti-flag by using One-step WB kit -M (a rapid 30-min incubation). Blots were developed with DAB.

Sup: Supernatant before loading to column,
Ft: Flow-through,
W: Wash fraction,
E1~E7: Eluted fractions 1~7.
Std: Standard, purified P50-flag with known concentrations (indicated on the left side).

III. Rapid Titration of Primary Antibody.

One-step WB offers a fast and simple means to titrate primary antibody to determine the optimal concentration of primary antibody for Western-blot. The method is also suitable to examine antibodies from various sources for selection of the best antibody.

30-minutes dot-blot-titration of primary antibody. Six serially-diluted antigen were dot-blotted (1µl/dot) onto six NC membranes (1.5cm x 2.5cm) as indicated on the right panel. Membranes were incubated for 30 minutes with 2ml Enhancer containing 40µl WB Probe-R and one of the following amounts of rabbit polyclonal antibodies: 0.5ul (panel 1), 1ul (panel 2), 2ul (panel 3), 3ul (panel 4), 4ul (panel 5) and 6ul (panel 6). Blots were developed with DAB.

Troubleshooting Guide:

Problem	Probable Cause	Solution
Signal is weak or invisible.	Too little protein is used.	Load more protein(s) onto the SDS-PAGE gel.
	Poor Western-transfer.	Optimize transfer time and/or the electrical current. Make sure that there are no air bubbles between the membrane and gel.
	Wrong type of primary antibody or One-Step WB Kit.	Check the label of the Kit and select a correct primary antibody compatible with One Step WB Kit. The kits labeled with -R, -M and –G are suitable for using with rabbit, mouse and goat primary antibodies, respectively.
	Poor specificity in binding activity of the primary antibody.	Use high affinity and purified monoclonal or affinity-purified polyclonal primary antibodies.
	Primary antibody diluted too much.	Increase the concentration of the primary antibody. Titration of primary antibody by Dot blot using serially diluted samples (from 1 µg/µl to 1 ng/µl) and serially diluted primary antibody is highly recommended to optimize primary antibody concentration.
	Primary antibody or One-Step WB Kit is defective.	Check Expire Date of the antibody as well as the One-step WB kit. Use fresh or unexpired antibody or kit.
	Incubation time too short or the reagent has not been warmed up.	Increase One-step Reaction time. In most cases, 30-minutes to one-hour incubation at room temperature is enough. However, when the antigen amount to be detected is very small or primary antibody is weak, increasing incubation time is helpful. Also, if solutions stored in a refrigerator are used before being pre-warmed to RT, longer incubation time is needed.
	Wash time too long.	Reduce the washing time.
High background, non-specific bands on blot.	Non-specific binding and cross-reactivity of primary antibody.	Select antigen-specific high affinitive primary antibodies. Purified monoclonal or affinity-purified polyclonal primary antibodies are preferred. Increase Enhancer-soaking time (before adding WB Probe and primary antibody).
	Used too much primary antibody or WB Probe.	Reduce the amount of primary antibody or the amount of WB Probe for One-step Reaction. Optimize the amount of antibody or WB Probe by dot-blot.
	Wash not sufficient.	Increase washing time.
	Signal development time too long.	Reduce the development time.
	Contaminated reagents or equipments.	Use clean equipments, freshly prepared buffers. Wear gloves and use clean forceps to handle membranes.
	Signal development reagent too sensitive.	Dilute the mixed substrate solution with 1-2 volume of distilled water. Use chromogenic development reagents, such as DAB, which is less sensitive but produce lower background than Chemiluminescent reagent.