Easy-WESTERN-II Super

Primary Antibody Detection Reagent for Western Blots

User Manual for High Sensitivity and Strong Signal Detection

Immediately after receiving the kit, read the section titled COMPONENTS AND STORAGE. It is IMPORTANT to store reagents under proper storage temperatures to prevent inactivation of reagents.

This manual is for the following kits:

Cat. #	Product Name	Components
BCL-EZS21	Easy-WESTERN–II Super basic	MAD reagent, Dilution buffer
BCL-EZS22	Easy-WESTERN–II Super Marker detector set	MAD reagent, Dilution buffer, Marker Detector
BCL-EZS23	Easy-WESTERN-II Super full set	MAD reagent, Dilution buffer, Mouse IgG Enhancer, Marker detector
BCL-EZS24	Easy-WESTERN-II Super Mouse enhancer set	MAD reagent, Dilution buffer, Mouse IgG Enhancer

The kit and its components are for **RESEARCH USE ONLY**, not for diagnostics or medical purposes.

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INTRODUCTION

Easy-WESTERN (EZW) is a primary antibody detection reagent kit for Western blots. The kit is based on the Multi-Antibody Detection (MAD) technology. The MAD reagent is nano-size protein particles with high affinity to antibodies. Each particle is composed of about 100 antibody-binding proteins and is labeled with 50 HRP molecules. Because of these properties, MAD reagent enables high sensitivity and quick detection of primary antibodies.

The Easy-WESTERN kit is ideal for high sensitivity, signal enhancement, and simultaneous detection of multi-antigens that is not possible with standard Western blot techniques.

Advantages

1. No need for secondary antibody - MAD reagent can detect most primary antibodies*
2. Higher signal for weakly expressed antigens
3. Enhance signal by easy reprobing – no stripping the membrane
4. Improve signal while using less primary antibodies

* MAD reagent may not work well with goat IgG. For best results use Mouse IgG Enhancer with mouse IgG1 primary antibodies. The performance of EZW depends on the type of antibody, and we do not warrant higher sensitivity in all cases.

COMPONENTS AND STORAGE

1. Multi-Antibody Detection (MAD) Reagent (250μL), store at -20°C immediately upon receipt and after every use.
2. 10x Dilution Buffer (60mL), store at 4°C after diluted
3. Marker Detection Reagent (50μL,kits BCL-EZS22, BCL-EZS23), store -20°C
4. Mouse Enhancer Reagent (250μL, kits BCL-EZS23, BCL-EZS24), store -20°C

Marker Detection Reagent and Mouse Enhancer Reagent are provided in an anti-freezing solution, so that they do not freeze even at -20°C.

All components should be stored at the recommended temperatures to prevent inactivation.

REAGENTS NEEDED, NOT PROVIDED

1. TBS-T (150mM NaCl, 10mM Tris-HCl, 0.1% Tween-20, pH 7.6)
2. Distilled water
3. HRP substrate such as DAB for chromogenic detection or luminol-based for chemiluminescence

REAGENT PREPARATION

1. Prepare TBS-T or purchase ready made.
2. Dilute 10x Dilution Buffer 1:10 with distilled water. This will make a working stock of 1x Dilution Buffer for the MAD reagent.
3. Dilute the 1x Dilution Buffer 1:10 with TBS-T. This will make a working stock of 1/10x Dilution Buffer for the

primary antibody

4. To detect molecular weight markers that are typically detected by secondary antibodies such as MagicMark XP, use Marker Detector reagent provided with kits BCL-EZS02 and BCL-EZS03. Dilution instructions provided with each protocol below.
5. To enhance weak signals from mouse IgGs, such as IgG1, use the Mouse Enhancer reagent provided with kits BCL-EZS03 and BCL-EZS04. Dilution instructions provided with each protocol below.

ASSAY PROTOCOLS

STANDARD PROTOCOL

This method is for high sensitivity and strong signal.

1. Separate protein sample(s) using SDS-PAGE
2. Transfer protein to PVDF membrane
3. Block with blocking solution for 1 hour at room temperature (RT).
   a. Ordinary blocking reagents, such as BSA based, casein-based blocking solutions and skim milk solutions, are all compatible with the kit. If you use skim milk, it has to be at reagent grade. Skim milk may sometimes give weaker signals.
4. Wash membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
5. Incubate the membrane with primary antibody in 1/10x Dilution Buffer for 1 hr at RT. Primary antibody should be diluted to manufacturer’s specifications.
6. Wash the membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
7. Dilute the MAD reagent 1:2,000 in 1x Dilution Buffer and incubate membrane in it for 1 hr at RT. To get stronger signal, use MAD reagent at a 1:1,000 dilution.
   a. If mouse IgG is used for the primary antibody, add the Mouse Enhancer reagent to the MAD containing Dilution Buffer at the volume of 1/2000 of the Dilution Buffer. The Mouse Enhancer reagent is included in kits BCL-EZS03 and BCL-EZS04,
   b. If molecular weight markers such as MagicMark XP are used, add the Marker Detector reagent to the MAD containing Dilution Buffer solution at the volume of 1/10000 of the Dilution Buffer. The Marker Detector reagent is included in kits BCL-EZS02 and BCL-EZS03.
8. Wash membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
9. Detect signal with commercially available HRP substrate.

REPROBING PROTOCOL

This method is to enhance weak signals without stripping the membrane to reprobe.

1. The membrane must still be wet with buffer from the original probing method. Dried membranes cannot be used.
2. Wash membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.

3. Dilute the MAD reagent 1:2,000 in 1x Dilution Buffer and incubate membrane in it for 1 hr at RT. To get stronger signals, use MAD reagent at a 1:1,000 dilution.
    a. If mouse IgG is used for the primary or 2nd antibody, add the Mouse Enhancer reagent to the MAD containing Dilution Buffer at the volume of 1/2000 of the Dilution Buffer. The Mouse Enhancer reagent is included in kits BCL-EZS03 and BCL-EZS04,
    b. Generally, the Maker Detector reagent is not needed when a 2nd antibody is originally used to probe the membrane. If a stronger marker signal is needed, add the Marker Detector reagent to the MAD containing Dilution Buffer solution at the volume of 1/10000 of the Dilution Buffer. The Marker Detector reagent is included in kits BCL-EZS02 and BCL-EZS03.
4. Wash membrane with TBS-T for 5 minutes. Repeat 4 more times for a total of 5 washes.
5. Detect signal with commercially available HRP substrate.

ENHANCED SIGNAL USING 2ND ANTIBODY PROTOCOL

This protocol is designed for using MAD to enhance signal from a 2nd antibody-HRP.

1. Separate protein sample(s) using SDS-PAGE
2. Transfer protein to PVDF membrane
3. Block with blocking solution for 1 hour at room temperature (RT).
4. Wash membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
5. Incubate the membrane with primary antibody diluted in any buffer you usually use for 1 hr at RT. Primary antibody should be diluted to manufacturer's specifications.
6. Wash the membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
7. Incubate the membrane in the 2nd antibody conjugated with HRP in any buffer you usually use for 1 hr at RT. Secondary antibody should be diluted to manufacturer's specifications.
8. Wash membrane with TBS-T for 5 minutes. Repeat 2 more times for a total of 3 washes.
9. Dilute the MAD reagent 1:2,000 in 1x Dilution Buffer and incubate membrane in it for 1 hr at RT. To get stronger signals, use MAD reagent at 1:1,000 dilution.
    a. If mouse IgG is used for the primary or 2nd antibody, add the Mouse Enhancer reagent to the MAD containing Dilution Buffer at the volume of 1/2000 of the Dilution Buffer. The Mouse Enhancer reagent is included in kits BCL-EZS03 and BCL-EZS04.
    b. Generally, the Maker Detector is not needed when a 2nd antibody was used to probe the membrane. If a stronger marker signal is needed, add the Marker Detector reagent to the MAD containing Dilution Buffer solution at the volume of 1/10000 of the Dilution Buffer. The Marker Detector reagent is included in kits BCL-EZS02 and BCL-EZS03.
10. Wash membrane with TBS-T for 5 minutes. Repeat 4 more times for a total of 5 washes.
11. Detect signal with commercially available HRP substrate.

TROUBLE SHOOTING

Problem	Possible Solutions
Weak signal	Increase antigen concentration
	Increase primary antibody concentration
	Increase the electric current or transfer time to improve protein transfer to membrane.
	Over blocking can reduce signal intensity. Reduce the blocking time or lower the concentration of blocking agents.
	Primary antibody is either mouse IgG or Goat IgG. Consider using kits BCL-EZS03 or BCL-EZS04 which contains Mouse Enhancer for improved signal detection of mouse IgG. EZS kits do not work well with goat IgG.
	When diluting MAD Reagent in buffer without blocking agents, use low protein binding tubes.
White out of luminescent signal	Too much antigen or antibody. Too much signal inhibits luminescence. Reduce the concentration of antigen or antibody used.
Too many extra-bands	Non-specific binding of primary antibody. Reduced the antibody to appropriate concentration.
	Too much protein. Reduce the amount of protein in electrophoresis.
	Too high a concentration of MAD Reagent. Reduce MAD in reaction.
	Insufficient blocking. Block the membrane with 5% skim milk in TBS-T for over 1 hour
	MAD reagent is inactivated due to inappropriate storage. MAD Reagent should be stored at -20°C. Inactivated MAD can produce non-specific signals. Replace MAD Reagent.
High background	Insufficient washing. Increase the number and the duration of washes.
	Adequate signal but with high background, decrease primary antibody concentration and or decrease incubation time.
	Reduce the concentration of MAD Reagent.
	When using antigen-antibody reaction enhancers, insufficient washing causes high background. Increase the number and the duration of washes.
Weak signal of 1 antigen when detecting multi-antigens.	One primary antibody weakly binds to antigen or MAD Reagent. Increase the concentration of the primary antibody giving the weak signal.
	Insufficient washing of primary antibody. Increase the number and the duration of washes.

Related products

Product #	Product name	description
BCL-EZM01	Marker detector	for Easy-WESTERN Kits, 50 test
BCL-EZE01	Mouse IgG enhancer	for Easy-WESTERN kits, 50 test
BCL-EZB21	10x Dilution buffer	for Easy-WESTERN Kits, 60mL
BCL-125A	Signal Booster Solution A	Enhancer for antibody-antigen reaction, 250 mL

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