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# Produktinformation

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## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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Elabscience®

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**（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）**

# Western Blot Detection Kit

**Catalog No:** E-IR-R304A
**Sizes:** 50 T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA)  240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## Introduction

Western Blot (WB) is the most common and classical experimental method for detecting protein expression in the biological science. However, due to differences in reagent sources, methods of operation and experience used by different Laboratory Technician, a large number of WB experimental results do not meet the requirement of paper publishing.

Elabscience has been focusing on the field of immunology for many years and has accumulated a lot of experience in WB operation. In order to help researchers solve the above problems, the Western Blot kit is now available. The reagents in this kit are provided for classical WB experiments, including all reagents needed from protein extraction to result detection, which have advantages of simple operation, high detection sensitivity, low background and strong system stability. And Elabscience provides detailed operation description and professional technical support. Additionally, it offers you convenience and guarantee to work out ideal Western Blot experiment results.

As the PVDF membranes have two pore sizes for different molecular weights of target proteins respectively. In order to meet different experimental requirements, we have designed two specifications of Western Blot kits.

E-IR-R304A (PVDF membrane, 0.45 μm) is recommended for the proteins detection with molecular weights more than 20 kDa.

E-IR-R304B (PVDF membrane, 0.22 μm) is recommended for the proteins detection with molecular weights less than 20 kDa.

## Components

| Product | Catalog No | Specification | Storage | Shelf life |
|---|---|---|---|---|
| RIPA Lysis Buffer (with PMSF and $Na_3VO_4$) | E-BC-R327 | 5 mL/50 μL+50 μL | -20 ℃ | 6 months |
| Total Protein Colorimetric Assay Kit (BCA method) | E-BC-K318 | 48 T | 2~8/-20 ℃ | 6 months |
| Excellent Chemiluminescent Substrate Detection Kit | E-BC-R347 | 15 mL/15 mL | 2~8 ℃ | 6 months |
| Pre-stained Protein Marker (10~180kDa) | E-BC-R273 | 25 μL | -20 ℃ | 12 months |
| Goat Anti-Rabbit IgG(H+L)-HRP | E-AB-1003 | 20 μL | -20 ℃ | 12 months |
| Skim Milk Powder | E-BC-R337 | 15 g | RT | 6 months |
| PBS Buffer, pH7.4 (10 ×) | E-BC-R187 | 100 mL | RT | 6 months |
| 5 X SDS Loading Buffer | E-BC-R288 | 1 mL×2 | -20 ℃ | 6 months |
| Electrophoresis Buffer (10 ×) | E-BC-R331 | 125 mL×2 | RT | 6 months |
| Transmembrane Buffer (10 ×) | E-BC-R333 | 125 mL×2 | 2~8 ℃ | 6 months |
| TBST Buffer (10 ×) | E-BC-R335 | 125 mL×2 | RT | 6 months |
| PVDF Membrane (0.45μm, 8.5×6cm) | E-BC-R266 | 5 pieces | RT | 12 months |

## Alternative Reagent offer

| Product | Catalog No | specification | Storage | Shelf Life |
|---|---|---|---|---|
| Goat Anti-Mouse IgG(H+L)-HRP | E-AB-1001 | 20 μL | -20 ℃ | 12 months |

## Self-Prepared Reagent

Methanol

## Instructions

**Preparation of Protein Samples**

1. **Protein Extraction**

   1） For Tissue Sample

   a. Take the samples, wash the tissue thoroughly with pre-cooled PBS Buffer (0.01 M, pH7.4) to remove the surface blood and internal debris.

   b. Weigh and smash the tissue, add an appropriate ratio of RIPA Lysis Buffer (E-BC-R327) (add 10 μL PMSF and 10 μL $Na_3VO_4$ to each 1 mL RIPA Lysis Buffer) and homogenizely lyse the tissue.

   It is recommended to homogenize according to the ratio of tissue weight: RIPA Lysis Buffer volume = 3:10. For example, add 1 mL RIPA Lysis Buffer to 0.3 g tissue sample, the specific volume can be adjusted according to experimental requirements.

   c. Shake and lyse on the ice for 30 min after homogenization. And then sonicate the sample for 1 min (under ice water bath conditions) with 2 s' sonication and 2 s' intervals to make cells fully lysis and reduce the viscosity of sample.

   d. Centrifuge at 12,000 rpm for 10 min at 4 ℃.

   e. Take the supernatant and measure the protein concentration mentioned in step2.

   2） For Cell Sample

   a. Collect the cells, wash them thoroughly with pre-cooled PBS Buffer (0.01 M, pH7.4) to remove the medium off (it is generally recommended to wash 3 times).

   b. Add an appropriate ratio of RIPA Lysis Buffer (10 μL PMSF and 10 μL $Na_3VO_4$ in each 1 mL RIPA Lysis Buffer) and lyse on the ice for 30 min.

   It is recommended to add 0.1 mL of RIPA Lysis Buffer to each well of a 6-well plates (the protein content in different cells may vary, and the volume of the lysate added can be appropriately adjusted).

   c. Sonicate the sample for 1 min (under ice water bath conditions) with 2 s' sonication and 2 s' intervals to make cells fully lyse and reduce viscosity of sample.

   d. Centrifuge at 12,000 rpm for 10 min at 4 ℃.

   e. Take the supernatant and measure the protein concentration mentioned in step2.

2. **Measurement of Protein Concentration**

By the BCA method (see the Total Protein Colorimetric Assay Kit (E-BC-K318) instructions).

3. **Adjust the Protein Concentration with PBS Buffer**

   Add 5 × SDS Loading Buffer (E-BC-R288) with the ratio of the protein sample: 5 × SDS Loading Buffer = 4:1 and boil the mixture for 10 min. Centrifuge at 12,000 rpm for 2 min and collect the supernatant. The denatured protein can be employed to Western Blot experiments or stored at -20 ℃ or -80 ℃.

**Tip:** It is recommended that the total protein loading amount of test sample is about 50 μg in each well. Try to make the loading volume of each sample close to 10 μL.

**Electrophoresis**

1. According to the molecular weight of the target protein, prepare appropriate separation gel. Add the test sample to each well, and add 5 μL of Pre-stained Protein Marker (E-BC-R273) to a reserved well in order to verify the target molecular weight and the extent of membrane transfer. Add Electrophoresis Buffer (E-BC-R331) and start electrophoresis.
2. Electrophoresis at 80v when the samples are in stacking gel, then convert to 120v when the blue flow into separating gel. Electrophoresis time is about 2~3 hours till bromophenol blue reaches the bottom of the gel.

**Transfer Membrane（Wet Transfer）**

1. Choose the PVDF Membrane (E-BC-R266) with a pore size of **0.45 μm** according to the molecular weight of the target protein. Soak the PVDF Membrane in methanol for 1 min to activate it, and then soak the PVDF Membrane in the Transmembrane Buffer (E-BC-R333), the filter paper and fiber mat must be soaked in the Transmembrane Buffer for use too.
2. Place the following materials in the order of the black plate (negative electrode) - fiber mat - filter paper - gel - PVDF Membrane - filter paper - fiber mat - white plate (positive electrode) are placed in order, discharge bubbles, clamp and place in the wet transfer tank. Adjustment the transmembrane conditions according to molecular weight of target protein. Make sure that the transmembrane process is carried out at low temperatures.

   Tip: This is for wet transfer. If other transmembrane methods are used, please adjust according to the specific conditions.
3. After the transmembrane, take out the PVDF Membrane carefully and wash with TBST Buffer for 1 min.

**Incubation of Antibodies**

1. Soak the PVDF Membrane with TBST Buffer (E-BC-R335) containing 5% Skim Milk Powder as blocking buffer and block the membrane at room temperature for 1.5 h.
2. According to the recommended primary antibody dilution ratio, use the TBST Buffer containing 5% Skim Milk Powder to dilute the primary antibody according to the antibody manual, soak the PVDF Membrane in the primary antibody working solution, incubate overnight at 4 ℃, and gently shake.
3. Wash the PVDF Membrane with TBST Buffer for 3 times, 15 min/ time.
4. According to the recommended secondary antibody dilution ratio, use a TBST Buffer containing

2% Skim Milk Powder to dilute secondary antibody (E-AB-1003, E-AB-1001) at 1:5000. Incubate at room temperature for 1 h on a shaker.

5. Wash the PVDF Membrane with TBST Buffer for 3 times, 15 min/ time.

**Detection**

1. Mix A and B in the Excellent Chemiluminescent Substrate Detection Kit (E-BC-R347) at the ratio of 1:1 as working solution.
2. Take out the PVDF Membrane from TBST Buffer and absorb the liquid with the filter paper. Pave the PVDF Membrane on the detection machine, add ECL working solution continuously on the PVDF Membrane, discharge the bubble and detect the result.
3. Adjust the contrast and the exposure time to get the best image.

## Cautions

1. This kit is for five gels (about 50 samples) with the Western Blot experiment.
2. Please store the reagents according to the storage conditions provided in the manual.
3. Most reagents in this kit are 10 × concentrate solution, please dilute them to 1 × working solution before use.
4. Keep the product sealed to prevent from pollution.
5. For your safety and health, please wear the lab coat and disposable gloves before the experiments.
6. For more details about the reagent using manual in this kit, please visit our website: www.elabscience.com