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

Western Blot Detection Kit

Catalog No: E-IR-R304A/E-IR-R304B

Product size: 50 Assays

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 (info in the header of each page).

Toll-free: 1-888-852-8623
TEL: 1-832-243-6086
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Email: techsupport@elabscience.com
Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.
Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Catalogue

Introduction

Elabscience® Western Blot Detection Kit has the characteristics of simple operation, high sensitivity, low background and strong stability. The kit contains reagents required for classical WB experiments, such as protein extraction reagents, ECL substrates, etc.

Detection Principle

Immunoblotting is used to identify macromolecular antigens (usually proteins) capable of interacting with specific antibodies, and to determine the size of the antigens. Proteins are first separated by SDS polyacrylamide gel electrophoresis, and then transferred to solid supports by electrophoresis, such as nitrocellulose membranes, polyvinylidene difluoride (PVDF) membranes, and cationic nylon membranes. Unreacted sites on the membrane are then blocked to inhibit non-specific adsorption of antibodies, so that the immobilized proteins can interact with specific polyclonal or monoclonal antibodies. Finally, localization is carried out by radiometric, chromophore or chemiluminescence methods.

Components

Catalog No	Product	Specification	Storage	Exp
E-BC-R327	RIPA Lysis Buffer (Strong)	5 mL	-20 ℃	12 months
E-BC-R287	100 mM PMSF	50 μL	-20 ℃	12 months
E-BC-R250	100 mM Na_3VO_4	50 μL	-20 ℃	12 months
E-BC-K318	BCA Protein Colorimetric Assay Kit	48 T	RT	12 months
E-BC-R288	5× SDS Loading Buffer	1 mL×2	-20 ℃	12 months
E-BC-R273	Pre-stained Protein Marker (10~180 kDa)	25 μL	-20 ℃	12 months
E-BC-R331	Electrophoresis Buffer (10×)	125 mL×2	RT	12 months
E-BC-R333	Transmembrane Buffer (10×)	125 mL×2	4 ℃	12 months
E-BC-R266/ E-BC-R329*	PVDF Membrane (0.45 μm,8.5 cm×6 cm)/ PVDF Membrane (0.22 μm, 8.5 cm×6 cm) *	5 pieces	RT	12 months
E-IR-R100	Filter paper	30 pieces	RT	12 months
E-BC-R337	Skim Milk Powder	15 g	RT	12 months
E-AB-1003	Goat Anti-Rabbit IgG (H+L) (peroxidase/HRP conjugated)	20 μL	-20 ℃	12 months
E-AB-1001	Goat Anti-Mouse IgG(H+L) (peroxidase/HRP conjugated)	20 μL	-20 ℃	12 months
E-IR-R307A	ECL Substrate A	15 mL	4 ℃	12 months
E-IR-R307B	ECL Substrate B	15 mL	4 ℃	12 months
E-BC-R187	PBS Buffer, pH7.4 (10×)	100 mL	RT	12 months
E-BC-R335	TBST Buffer, pH7.4 (10×)	125 mL×2	4 ℃	12 months

* E-IR-R304A correspond to PVDF membrane (0.45μm) (Cat. No.: E-BC-R266). PVDF membrane (0.45μm) is suitable for proteins with molecular weight greater than 20KD.

E-IR-R304B correspond to PVDF membrane (0.22μm) (Cat. No.: E-BC-R329). PVDF membrane (0.22μm) is suitable for proteins with molecular weight less than 20KD.

Materials Not Supplied

Methanol

Instructions for use

This kit can complete Western Blot experiments corresponding to 5 gels (except Marker, which can detect 45 samples).

Precautions

1. This kit is only for scientific research and cannot be used in clinical experiments.
2. After receiving the kit, please store the regents according to the storage conditions in the manual.
3. For your safety and health, please take safety precautions and follow the procedures of laboratory reagent operation.

Protein Extraction

1. Reagent preparation:

1×PBS: PBS Buffer, pH7.4 (10×) (E-BC-R187) is a 10× concentrated solution, diluted with deionized water to 1× PBS before use.

RIPA Lysis working solution: Add 10 μL PMSF (E-BC-R287) and 10 μL Na_3VO_4 (E-BC-R250) to 1 mL RIPA Lysis Buffer (E-BC-R327) immediately before use.

2. Procedure

1) **For Tissue Sample:**

a. Collect the tissues, wash the tissues three times with cold 1× PBS to remove the surface blood and internal debris.

b. Weigh and smash the tissue, add RIPA Lysis working solution and homogenizely lyse the tissue. Shake and keep on the ice for 30 min after homogenization.

Tip: Add 1 mL of RIPA Lysis working solution to 0.3 g of tissue samples, and the ratio can be adjusted according to experimental requirements.

c. Sonicator 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 viscosity of sample.

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

e. Transfer supernatant to a new tube for further analysis.

2) **For Cell Sample:**

a. Collect the cells, wash cells three times with cold 1× PBS.

b. Add cold RIPA Lysis working solution to the cells. Shake and keep on ice for 30 minutes.

Tip: Use 1mL of cold RIPA Lysis working solution for every 1×10^7 of cells (the protein content in different cells may vary, and the volume of RIPA Lysis working solution added can be adjusted appropriately).

c. Sonicator 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 viscosity of sample.

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

e. Transfer supernatant to a new tube for further analysis.

Measurement of Protein Concentration

Detect the protein concentration by the BCA method, please see the instructions of Total Protein Colorimetric Assay Kit (E-BC-K318).

SDS-PAGE

1. **Reagent preparation:**

   1× Electrophoresis working Buffer: dilute Electrophoresis Buffer (10×) (E-BC-R331) with deionized water for 10 times before use.

2. **Materials Not Supplied**

   SDS-PAGE Gel

3. **Procedure**

1) Dilute the protein sample (4:1) in 5× SDS Loading Buffer (E-BC-R288) to yield 1× SDS protein solution, and boil the mixture at 95-100 ℃ for 5-10 min.
2) Centrifuge for 2 min at 12,000 rpm at 4 ℃ in a microcentrifuge, and collect the supernatant.

Tip: Heating at 70 ℃ for 5-10 min is also acceptable and may be preferable when studying multi-pass membrane proteins.

3) According to the molecular weight of the target protein, prepare appropriate SDS-PAGE Gel (self-prepared). Pour enough 1× Electrophoresis working Buffer into the unit to fill the buffer chamber and completely cover and submerge the gel.
4) Carefully remove the comb by tapping lightly to loosen, and slowly lifting straight up out of the gel tray to avoid damage to the wells.
5) Load equal amounts of protein into the wells of the SDS-PAGE gel, along with Pre-stained Protein Marker (E-BC-R273).

Tip: Load 30–50 μg of total protein from cell lysate or tissue homogenate, or 10–100 ng of purified protein. Add a 10 ul volume of total protein to one SDS-PAGE gel well.

6) Place on lid and attach red and black cables to power supply.
7) Turn on power supply, run the gel at 80 V constant. When the bromophenol blue indicator moves to the junction of the stacking gel and the separating gel

to form a line, change to a constant voltage of 120 V.

8) When the gel run is complete and tracking dye has migrated as far through the gel as desired, or to the end of the gel, turn off the power.

Transfer Membrane（Immersion method）

1. Reagent preparation:

1× Transfer Buffer：Add 100 mL of Transmembrane Buffer (10×) (E-BC-R333) and 200 mL of methanol (materials not supplied) to 700 mL of deionized water and mix fully.

Tip: The recommended ratio of methanol in 1× Transfer Buffer is 20%, which can be adjusted according to the specific situation.

1× TBST Buffer: dilute TBST Buffer, pH7.4 (10×) (E-BC-R335) with deionized water for 10 times before use.

2. Materials Not Supplied

Methanol

Fiber mat

3. Procedure

1) Cut PVDF membrane (E-BC-R266/E-BC-R329) to the same dimensions of the gel. Cut a notch in the membrane corner to correspond to a corner of the gel.
2) Wet PVDF membrane in 100% methanol for 1 min.
3) Place PVDF membrane in a new container with 1× Transfer Buffer and equilibrate for 15 to 20 minutes.
4) Wet the filter paper (E-IR-R100) in 1× Transfer Buffer.
5) Remove gel from the electrophoresis apparatus and equilibrate in 1× Transfer Buffer for 30 minutes with gentle shaking.
6) Place pre-soaked transfer membrane on the pre-soaked gel. Remove air bubbles by rolling a glass pipette over the membrane surface.
7) Place the pre-soaked filter paper on top of the transfer membrane. Remove any trapped air bubbles.
8) Place 2 soaked blotting pads into the cathode (–) core of the blot module.
9) Place the following material in the order of the black plate (negative electrode)

- fiber mat - filter paper - gel - PVDF membrane - filter paper - fiber mat - white plate (positive electrode) in order.

10) Fill the blot module with 1× Transfer Buffer until the gel/membrane sandwich is covered in 1× Transfer Buffer.
11) Connect the leads and perform transfer for 30-120 minutes at 150-300 mA.

    Tip:

    a) Transfer time and electric current will vary depending upon polyacrylamide concentration, gel thickness, the presence of SDS or methanol, pH and ionic strength of the 1× Transfer Buffer and the molecular weight of the protein. The transfer conditions need to be confirmed according to the specific conditions of the experiment.

    b) If other transmembrane methods are used, please adjust according to the specific conditions.

12) When the transfer is complete, disconnect leads and disassemble the transfer stack to remove the membrane.
13) Wash with 1×TBST Buffer for 1 min. Keep membrane moist until ready to use.

Immunodetection

1. Reagent preparation:

Blocking Buffer： 1×TBST Buffer containing 5% Skim Milk Powder (E-BC-R337) can be used as a blocking buffer.

2. Procedure

1) Place the PVDF membrane in Blocking Buffer at room temperature for 90 minutes with shaking (1 revolution/sec).
2) Add diluted primary antibody solution with 5% Skim Milk Powder. Incubate overnight at 4℃ with shaking.
3) Add enough 1×TBST Buffer and wash with shaking for 15 minutes, then pour off. Repeat this step 3 times.
4) Add diluted secondary antibody solution with 2% Skim Milk Powder. Incubate for 60 minutes.
5) Add enough 1× TBST Buffer and wash with shaking for 15 minutes, then decant. Repeat this step 3 times.

Tip：

a) E-AB-1001 is peroxidase-labeled goat anti-mouse IgG, corresponding to the use of mouse primary antibody;
b) E-AB-1003 is peroxidase-labeled goat anti-rabbit IgG, corresponding to the use of rabbit primary antibodies.
c) Skim milk powder contains trace amounts of biotin and is not suitable for biotin-avidin detection systems.
d) Choose the appropriate blocking buffer according to different experimental systems.

Detection

1. **Reagent preparation:**

   ECL Working Solution **:** Mix equal parts of the ECL Substrate A (E-IR-R307A) and ECL Substrate B (E-IR-R307B). For best results, use the working solutions immediately after mixing.

2. **Procedure**

1) Lay the blot on a clean piece of plastic sheet (such as clear copier transparency material), antigen side up. Please keep the membrane moist.
2) Pipet ECL Working Solution onto the blot surface for 1-5 min, taking care to cover the entire blot.
3) Absorb excess substrate by gently blotting with clean absorbent material (such as filter paper or tissue) or by tilting the plastic sheet so excess substrate drains off onto the absorbent material. Meanwhile, discharge the bubble.
4) Place the protected blot in a film cassette or imaging system and expose for 1 second to several minutes as desired.

Cautions

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

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

Total Protein Assay Kit (With standard, BCA method)

Catalog No: E-BC-K318

Product size: 48T

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 (info in the header of each page).

Toll-free: 1-888-852-8623
TEL: 1-832-243-6086
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Website: www.elabscience.com

Please refer to specific expiry date from label outside of box.
Please kindly provide us with the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can measure protein content of a variety of animal serum, plasma, culture cells, tissue and other samples.

Detection principle

Cu^{2+} can be reduced to $Cu^{+}$by protein in alkaline condition. Cu^{+}can combine with BCA reagent and form purple complex, which has a maximum absorption peak at 562 nm. The absorbance value is proportional to the protein concentration. Therefore, the protein concentration can be calculated according to the OD value.

Components

Item	Component	Specification
Reagent 1	BCA Reagent	10 mL×1 bottle
Reagent 2	Copper Salt Solution	250 μL×1 vial
Reagent 3	Protein BSA Standard	0.563 mg×1 vial

Storage

Store at room temperature for 12 months.

Reagent preparation:

1. Preparation of 563 μg/mL protein standard

   Add 1 mL of 0.01 M pH 7.4 PBS to Reagent 3, dissolve and mix well. Store on ice until use, and store in aliquots at -20 ℃ for 3 months.

2. Preparation of BCA working solution

   Mix well according to the volume ratio of reagent 1: reagent 2 to 50:1. The prepared BCA working solution can be stored at 2-8 ℃ for 24 hours. It is recommended to use it immediately after the preparation is completed.

Procedure

1. blank well: Take 20 μL of normal saline or phosphate buffer solution and add it to the corresponding blank well

   Standard well: Add 20 μL of standard to the corresponding standard wells;

   Sample well: Take 20 μL of the sample to be tested and add it to the corresponding sample well.

2. Add 200 μL of BCA working solution to the wells of Step 1
3. Oscillate for 20 s to mix fully and incubate at 37℃ for 30 min.
4. Measure the OD value of each well at 562 nm with microplate reader

Tip: When the reagent is added to the ELISA well, it should be added to the bottom of the ELISA plate; the sample should be added slowly to avoid the generation of air bubbles. (Bubble will affect the measurement result)

5. Eration table

	blank well	Standard well	Sample well
normal saline or phosphate buffer solution（μL）	20		
563 μg/mL Standard（μL）		20	
Samples (μL)			20
BCA working solution(μL)	200	200	200
Oscillate for 20 s to mix fully and incubate at 37℃ for 30 min. Measure the OD values of each well at 562 nm with microplate reader.			

6. protein concentration(μg/mL)= $\frac{a-c}{b-c} * d * f$

Note:

a: The absolute OD value of Sample

b: The absolute OD value of standard

c: The absolute OD value of blank

d: The concentration of standard

f: Dilution factor of sample before test

Technical parameter

1. The sensitivity of the kit is 16.5 μg/mL.
2. The intra CV is 2.2% and the inter CV is 4.5%.
3. The recovery of the kit is 100%.
4. The detection range of the kit is 16.5-1000 μg/mL.

Sample pretreatment

1. Serum sample:

Fresh blood was collected and placed at 25℃ for 30 min to clot the blood.Centrifuge the sample at 4℃ for 15 min at 2000 ×g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection. If not detected on the same day, stored the serum at-80℃, which can be stored for a month.

2. Plasma sample:

The fresh blood was added into the test tube containing anticoagulant and mixed upside down.Centrifuge the sample at 4℃ for 10 min at 700~1000 ×g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection. If not detected on the same day, stored the serum at-80℃, which can be stored for a month.

3. 10% tissue homogenate sample:

Accurately weigh the tissue sample, add 9 times the volume of PBS (0.01 M, pH7~7.4) according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

4. Culture cell sample:

Wash the cells with PBS (0.01 M, pH7~7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add PBS at a ratio of cell number (10 6): PBS (μL) =1: 300-500. Sonicate or grind with hand

operated in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection. Meanwhile, determine the protein concentration of supernatant (E-BC-K318, E-BC-K168, E-BC-K165).

Note:

The detection ranges is from 16.5 to 1000μg/ml, please take 1-2 preliminary experiments before sample tests, and then diluted the samples with normal saline.

Recommended dilution multiple:

Serum (plasma) sample: 50-70 times;

Animal tissue homogenate: 8-12 times;

Plant tissue homogenate: 3-6 times;

Cell sample: 2-3 times.

Cautions

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. Do not use components from different batches of kit.
4. Control the incubation time strictly.
5. Prevent the formulation of bubbles when adding the reagents to the microplate.