Universal WB Toolkit

Cat #: KTD106-EN **Size:** 20 T

	Universal WB Toolkit		
REF	**Cat #:** KTD106-EN	LOT	**Lot #:** Refer to product label
	Applications: Animal and Plant Tissues, Cells, Bacteria		
	Storage: Store according to the recommended storage conditions of each component, stable for 12 months		

Assay Principle

Western blotting is a method for detecting a certain protein in complex samples based on the specific binding of antigen and antibody. Due to the high resolution of SDS-PAGE and the high specificity and sensitivity of solid-phase immunoassays, western blotting has become a routine technique for protein analysis. Based on the tedious and time-consuming operation of traditional WB experiments, high background, and poor stability of experimental results, Abbkine has developed a Universal WB Toolkit. The toolkit contains: ①Optimized lysate for sample WB needs; ②Easy to operate, ready-to-use reagents, no need to prepare complex reagents, you can start the experiment right out of the box; ③A ready-to-use WB positive control, which can be directly loaded to provide a real WB experimental reference; ④SuperKine™ West Femto Maximum Sensitivity Substrate provides a highly sensitive color developing solution to enhance the chemical signal of immunological experiments.

Materials Supplied and Storage Conditions

Kit components		Size (20 T)	Storage conditions
Part 1 of 2	Lysis Buffer	2 mL	4℃
	Running Buffer (10×)	100 mL	4℃
	Transfer Buffer (10×)	100 mL	4℃
	TBST (10×)	100 mL	4℃
	BSA	3 g	4℃
	PVDF Membrane (8.5 cm×6 cm)	2	RT
	SuperKine™ West Femto Maximum Sensitivity Substrate A	4 mL	4℃, protected from light
	SuperKine™ West Femto Maximum Sensitivity Substrate B	4 mL	4℃
Part 2 of 2	WB Positive Control	40 μL	-20℃
	Anti-GAPDH Mouse Monoclonal Antibody (2B5)	6 μL	-20℃
	Colorcode Prestained Protein Marker (10-180 kDa)	5 μL	-20℃
	HRP, Goat Anti-Mouse IgG	3 μL	-20℃
	HRP, Goat Anti-Rabbit IgG	3 μL	-20℃

	SDS-PAGE Loading Buffer (5×)	1 mL	-20℃

Materials Required but Not Supplied

- Gel suitable for the concentration of target protein
- Methanol
- Filter paper
- Primary antibody
- Electrophoresis chamber, trans-blot transfer
- Water bath or metal bath, shaker

Reagent Preparation

Lysis Buffer: Denatured protein Lysis Buffer, extract protein for WB samples. Ready to use as supplied. Place it on ice for use. Store at 4℃.

1×Running Buffer: Prepare before use, dilute Running Buffer (10×) 10 times with deionized water. Store at 4℃.

1×Transfer Buffer: Prepare before use, take 100 mL Transfer Buffer (10×), add 200 mL anhydrous methanol, mix well, then add deionized water to 1 L. Store at 4℃.

1×TBST: Prepare before use, dilute TBST (10×) 10 times with deionized water. Store at 4℃.

Blocking Buffer, Primary/Secondary Antibody Dilution Buffer: Prepare before use, add 15 mL 1×TBST per 0.45 g BSA, and mix well.

Note: The concentrations of Running Buffer (10×), Transfer Buffer (10×) and TBST (10×) are high, which may lead to the precipitation of crystals in the liquid due to low temperature and other reasons. It is recommended to dissolve it in 37℃ water bath before use.

WB Positive Control: Ready to use as supplied. It can be directly used as an animal positive control in WB experiments.

Anti-GAPDH Mouse Monoclonal Antibody (2B5): The most commonly used loading control monoclonal antibody of GAPDH, the molecular weight of the target protein was 37 kDa. The recommended dilution ratio is 1:5,000.

Colorcode Prestained Protein Marker (10-180 kDa): Colorcode Prestained Protein Marker (10-180 kDa) contains 3 colors (blue, orange and green), is a mixture of 10 stained protein standards (10, 17, 25, 33, 40, 53, 70, 95, 130, 180 kDa). The Marker contains one orange reference band at 70 kDa and one green band at 10 kDa. The recommended loading volume is 2.5 μL.

HRP, Goat Anti-Mouse IgG: Horseradish Peroxidase labeled Goat Anti-Mouse IgG Secondary Antibody. The recommended dilution ratio is 1:10,000.

HRP, Goat Anti-Rabbit IgG: Horseradish Peroxidase labeled Goat Anti-Rabbit IgG Secondary Antibody. The recommended dilution ratio is 1:10,000.

SDS-PAGE Loading Buffer (5×): Ready to use as supplied. Store at -20℃.

Assay Procedure

A. Preparation of protein samples

1. Extract protein for Cell Samples:

(1) Collect cells (Adherent cells: 80% to 90% of monolayer cells were grown in a 10 cm cell culture dish. Remove the medium and wash with PBS once; Suspended cells: Collect 0.5-1×10^6 cells by centrifugation and washed by PBS once.

(2) Add 50-100 μL ice-cold Lysis Buffer to cells, the pipette is used to blow the mixture repeatedly, transfer cell suspension to a new tube.

(3) Centrifuge at 12,000 rpm for 3-5 min at 4℃, collect the supernatant.

2. Extract protein for Tissue Samples:

(1) Tissue Samples: Weigh 10 mg of tissue and add 100 μL Lysis Buffer, Homogenize tissue with dounce homogenizer. (If the protein concentration is low, reduce the volume of Lysis Buffer).

(2) Transfer the homogenate to a new tube, lytic samples at 4℃ for 1-2 min.

(3) Centrifuge at 12,000 rpm for 3-5 min at 4℃, collect the supernatant.

3. Extract protein for Bacteria Samples:

(1) Collect bacteria by centrifugation (1,2000 rpm for 2 min at 4℃) and washed by PBS once.

(2) Per 1 mL bacteria add 100 μL Lysis Buffer, Ultrasonic break in ice 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times).

(3) Centrifuge at 12,000 rpm for 3-5 min at 4℃, collect supernatant.

4. According to the SDS-PAGE Loading Buffer (5×) :Protein Sample=1:4, add SDS-PAGE Loading Buffer (5×) to protein samples, mixed well and boiled for 10 min. Centrifuge at 12,000 rpm for 3-5 min at 4℃. Take appropriate volume supernatant for sample loading.

Note: (1) Protease inhibitors are not necessarily added, but if downstream experiments take longer or are preserved for longer periods after protein extraction, it is advisable to add protease inhibitors. The BCA kit is recommended for protein concentration determination (Abbkine Cat #: KTD3001). To study protein phosphorylation, phosphatase inhibitors should be added to the cell lysis buffer before use. (2) If the protein concentration is low, increase the number of samples or reduce the volume of Lysis Buffer. (3) If weak protein bands in the high molecular weight range (100-300 kDa), increase the volume of Lysis Buffer to ensure adequate lysis.

B. Preparation of SDS-PAGE Gel

Preparation of different concentrations of separating gel (under gel), according to the molecular weight of the target protein. After the separating gel is solidified, 5% stacking gel (upper gel) is prepared. The following is for reference only.

Molecular weight of target protein	Concentration of separating gel (under gel)
<10 kDa	15%
10-30 kDa	12%
30-70 kDa	8%
>70 kDa	6%

C. Electrophoresis

Load protein samples and Colorcode Prestained Protein Marker onto SDS-PAGE gel, add 1×Running Buffer to Electrophoresis Chamber, Electrophoresis at 90 V for 30 min after bromophenol blue electrophoresis to separating gel, then adjust the voltage to 120 V until the end of electrophoresis.

Note: Precast PAGE Gel need to use its special electrophoresis Buffer, not the Running Buffer provided with this toolkit.

D. Transfer Membrane (for example, Wet Transfer)

The PVDF Membrane is soaked in methanol for 1-2 min to activate it, and then the PVDF Membrane, filter paper and sponge pad are soaked in the 1×Transfer Buffer. Prepare sandwich clips according to black plate (negative pole)-sponge sad-filter paper-gel-PVDF Membrane-sponge pad-red plate (positive pole), then get rid of air bubbles, turn into the (wet) Trans-Blot Transfer, add Transfer Buffer to Trans-Blot Transfer. The membrane was transferred under ice bath condition, the transfer voltage and time can be adjusted according to the target protein molecular weight.

E. Membrane Blocking

After transfer, wash PVDF Membrane with 1×TBST for 1 min, then Incubate PVDF Membrane in 15 mL of Blocking Buffer with gentle agitation for 1 h at room temperature.

F. Wash

Wash 3 times for 5 min each with 1×TBST.

G. Primary Antibody Incubation

Incubate PVDF Membrane with 15 mL diluted Primary Antibody (at the appropriate dilution ratio as recommended in the product datasheet), and then incubate on a shaker for 1-2 h at room temperature or overnight at 4℃.

H. Wash

Wash 3 times for 5 min each with 1×TBST.

I. Secondary Antibody Incubation

Incubate PVDF Membrane and secondary antibody (Select an appropriate secondary antibody according to the type of primary antibody) in 15 mL Antibody Dilution Buffer with gentle agitation 1-2 h at room temperature.

J. Wash

Wash 3 times for 5 min each with 1×TBST.

K. Develop and Expose

(1) Preparation of ECL Working Solution: mix the two reagents (SuperKine™ West Femto Maximum Sensitivity Substrate A and SuperKine™ West Femto Maximum Sensitivity Substrate B) at 1:1 ratio to prepare the ECL Working Solution.

(2) Clamp the membrane with tweezers, drying the water with filter paper, then soak the PVDF Membrane in the ECL Working Solution for 1-2 min. Expose to X-ray film or take photos by chemiluminescence imager.

Strawberry moment: Universal WB Toolkit can easily detect protein expression and make your experimental results more in line with experimental literature publication standards. The single product (KTD3003/BMM3001/BMU102-EN/A21010/A21020) can also be used for western blotting (WB) experiments. Scan the QR code on the right and follow the Abbkine official account to learn more about Abbkine products.

Typical Data

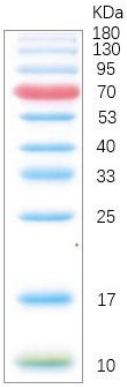

Figure 1. Image is from a 15% Tris-glycine gel (SDS-PAGE) transferred to membrane using Abbkine Colorcode Prestained Protein Marker (10-180 kDa). Loading volume: 5 μL.

Recommended Products

Catalog No.	Product Name	Recommended reason
KTD3003	SDS-PAGE Protein Sample Loading Buffer (5×)	Using a reducing agent with better stability, better reduction effect and no unpleasant odor
BMM3001	Colorcode Prestained Protein Marker (10-180 kDa)	Marker is a mixture of ten (10) blue, orange and green stained proteins (10, 17, 25, 33, 40, 53, 70, 95, 130, 180 kDa) for use as size standards in SDS-PAGE and WB
BMU102-EN	SuperKine™ West Femto Maximum Sensitivity Substrate	Providing a highly sensitive color developing solution to enhance the chemical signal of immunological experiments
A21010	HRP, Goat Anti-Mouse IgG	Affinity purified using solid phase Mouse IgG (H&L) with finally > 95% purity based on SDS-PAGE
A21020	HRP, Goat Anti-Rabbit IgG	Affinity purified using solid phase Rabbit IgG (H&L) with finally > 95% purity based on SDS-PAGE

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.