Western Blot Detection Kit

Cat.No.：RK05873P

Size：50 T

Product introduction

Western blotting is an experimental method commonly used in cell and molecular biology.

In this technique, biological samples processed by gel electrophoresis are stained with specific antibodies; The expression of specific proteins in the sample is analyzed by the stained bands. This kit is used for western blot detection.

Packing list of the Kit

Cat.No	Description	Size	Storage condition
RK05873N-1	SDS-PAGE Sample Loading Buffer (5*)	1mL*5	-20℃
RK05873N-2	ColorMixed Protein Marker 180 (10-180 kDa)	250μL*2	-20℃
RK05873N-3	SDS-PAGE Running Buffer (1*)	1L*7	RT
RK05873N-4	Western Transfer BufferBuffer (1*)	1L*8	RT
RK05873N-5	Membrane (PVDF)	0.2 μm (50pcs)	RT
RK05873N-6	Western Blocking BufferBuffer (1*)	250mL*2	4℃
RK05873N-7	TBST BufferBuffer (20*)	65mL*2	4℃
	Product instruction	1 copy	

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn

Storage condition:

Check the label information for each component, and store under recommended conditions.

Instruction

Experiment instruments & reagents

Sample Preparation Reagents

a. RIPA lysis buffer

b.Protease inhibitors

c. BCA working reagents

d. BSA Standard Solution (5mg/mL)

e. 1×PBS buffer

f. SDS-PAGE Sample Loading Buffer (5*)

（2）Gel Reagents

a．30% Acr-Bis (29:1)

b．1 M Tris-HCl (pH6.8)

C．1.5M Tris-HCl (pH8.8)

d．10%SDS

e．10%Ammonium persulfate (APS)

f．TEMED

（3） Electrophoresis, membrane transfer reagents and consumables

a.SDS-PAGE Running Buffer (1*)

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn

Pour an individually packaged 1L SDS-PAGE electrophoresis solution powder into a clean beaker, add about 800ml of distilled water and dissolve. Then make up to 1L in a graduated cylinder. Ready to use after mixing.

b.Western Transfer Buffer(1*)

Pour an individually packaged 1L Western Transfer Buffer powder into a clean beaker, add about 600ml of deionized water and dissolve. Add 200ml of absolute ethanol or 210ml of 95% ethanol, mix well, then make up to 1L with deionized water in a graduated cylinder. Mix well and it's ready to use.

c.Filter Paper (7.5×10cm)

d.Membrane

(4) Blocking, primary and secondary antibody incubation and exposure reagents

a.1XTBST Buffer

b.Western Blocking Buffer(1*)

1) Pour a packaged 250ml 1× TBS powder into a clean beaker, add about 150ml of distilled water and then add 250 ul of tween-20 to dissolve it. Adjust pH to 7.6 with HCl, mix well, and make up to 250 ml with deionized water in a graduated cylinder, mix again and ready to use.
2) Take a clean beaker with a scale, weigh 7.5g of BSA into the beaker, make up to 250ml with the prepared TBST solution (see step (1)), dissolve to make it as 3% BSA working solution.

   Note: Unused working solution should be used within 3 days.
3) After membrane transfer, rinse the membrane with Western Wash Solution for 1-2 min. Above, add 3% BSA working solution (See step (2)) and blocking for 60 min.
4) 20 min before the end of blocking, dilute the primary antibody with above 3% BSA working solution (See step (2)). E.g. For 1:1000primary antibody dilution ratio, add 1 ul of primary

antibody to each 1 ml of 3% BSA working solution. Mixing well and ready to use.

c. Skimmed milk powder

d. Bovine Serum Albumin (BSA)

e. Western Antibody Dilution Buffer

f. Secondary ab-HRP

g. ECL Reagent

Experimental procedures

1. Sample preparation

（1）Cell collection

a. Adherent cell culture collection

Remove the culture medium of adherent cells, rinse once with PBS, NS or serum-free medium, centrifuge at low speed, discard the supernatant, and save the pellet.

b. Suspension cell culture collection

Suspend the cells by speed centrifugation, then discard the supernatant, and collect the pellet. Flick the cells with your fingers to loosen them

c. Tissue samples collection

Cut the tissue into small pieces (the smaller the better).

Freeze the tissue in liquid nitrogen or ultra-low temperature freezer for > 30 min, and then quickly grind the tissue with liquid nitrogen within 1~2min in order to reduce the protein degradation.

（2）Total protein extraction

a. Cell / tissue lysis

Fully immerse the container with cell precipitates or tissue fragments in ice.

Add the corresponding volume (3mL if have sufficient cell mass, recalculate if not sufficient) of lysate to the cell precipitates at the ratio of 1mL lysate/10^7 cells (one T-75 flask), and lyse for 20min (Note: place the tube on a vortexer for 10 sec every 5 minutes).

Tissue fragments were added to the homogenizer as the ratio of 0.5 mL of lysed solution per 100 mg of tissue protein lysed solution, ground every 3 min, and repeated 5 times to grind the tissue as much as possible (Note: (Protease inhibitors can be added or not added to the lysate as needed).

b. Centrifugation

Balance the lysed sample, and then place it in a pre-cooled high-speed refrigerated centrifuge, centrifuge for 15 min at 12000 rpm.

c. Protein denaturation

After centrifugation, the supernatant will be the protein extract.

Pipette a small amount of protein extract for protein concentration determination. Add 1/5 of the supernatant volume of 5×Loading Buffer ((The final working solution is 1X) to the centrifuge tube of remaining protein extract.

While the temperature of the dry bath rises to 95°C, insert a 1.5mL centrifuge tube into the heating hole, proceed the heat denaturation at 95°C for 10min, then store at -20°C after the liquid is completely cooled.

(3) Protein concentration determination (BCA)

a. Prepare BCA working solution

According to the number of samples, add 50 volumes of BCA reagent A to 1 volume of BCA reagent B (50:1) to prepare an appropriate amount of BCA working solution, and mix well. BCA

working solution is stable within 24h at room temperature.

b. Standard sample determination

Take 10 μL of protein standard (5mg/mL BSA) and dilute to 50 μL, to make a final concentration of 1mg/mL.

The diluted protein standard can be stored at -20°C for a long time.

Note: Deionized water or 1*PBS can be used as a diluent for this standard solution.

Add 0, 1, 2, 4, 8, 12, 16, and 20 μL of the standard solution to the 96-well plate, respectively, and then add the diluent to make up to 20 μL (See attached table).

An appropriate amount of sample should be added to the sample wells of the 96-well plate. If the sample is less than 20 μL, diluent should be added to make up to 20 μL.

Record the sample volume.

Add 200 μL of BCA working solution to each well and let stand at 37 °C for 20-30 min.

Use a microplate reader to measure the absorbance of A562, or other wavelengths between 540-595nm.

Calculate protein concentration of the sample by the standard curve and the sample volume used.

2. Gel electrophoresis

(1) Gel-maker installation

Please see instruction manual.

(2) Preparation of the separating gel

According to different protein sizes, choose different concentrations of separating gels (See attached table). Pour the prepared gel solution into the installed glue maker and add isopropyl alcohol to seal the gel. Then place it horizontally at room temperature for about 30 min.

Note:

Prevent the bubbles generated by the gel solution from being injected into the gel maker; Add TEMED before injection to prevent solidification;

Isopropanol are injected by dragging slowly along the side of the glass plate.

(3) Preparation of the stacking gel

After the separating gel has solidified, pour the isopropyl alcohol along the side of the glass plate, and dry with filter paper. Then, prepare the stacking gel by required volume (See attached table). Inject the prepared gel solution into the gel maker, and slowly insert the prepared sample comb into the gel solution along one end of the glass plate, and let it stand horizontally at room temperature for 20-30min.

Note: Avoid air bubbles between the sample comb and the gel solution.

(4) Load samples

After the stacking gel has solidified, pull out the sample comb vertically with both hands.

Inject Tris-Glycine SDS Running Buffer into the inner and outer tanks, to form closed loop.

Use a pipette to get the sample, and load the sample vertically from the comb hole. The protein content is 25ug/well.

Note: The inner tank of Tris-Glycine SDS Running Buffer need be filled up, and the outer tank of Tris-Glycine SDS Running Buffer should be 3~5cm below the bottom.

(5) Electrophoresis

After loading the sample, connect the power of electrophoresis apparatus, and ensure that positive and negative poles are connected in the right side.

The electrophoresis parameter of the concentrated gel is set to a constant voltage of 80V. Can

increase it to 120V when proteins enter the separation gel.

Stop electrophoresis when bromophenol blue reaches the bottom of the gel and turn off the power of electrophoresis apparatus.

3. Protein transfer

（1）Preparation

a. Transfer buffer should be pre-chilled in a -20°C freezer at least 2h in advance (right after electrophoresis starts).

b.Cut the Filter Paper and Nitrocellulose membrane to the appropriate size.

c. If target protein >20KD, then select 0.45μm NC membrane.

If target protein <20KD, then select 0.2μm NC membrane or 0.22μm PVDF membrane.

After selection, soak the NC membrane in Western Transfer Buffer for later use.

Note:

If PVDF membrane is used, soak in methanol for 5-10 min, then soak in Western Transfer Buffer for later use.

（2）Protein transfer

Take out the gel from the glass plate, and sequentially place 1 porous pad, 3 filter papers, gel, NC membrane, 3 filter papers, and 1 porous pad on the splint as a “Sandwich” structure. Put the transferred membrane in the transfer tank, and transfer membrane under certain conditions. (See attached table)

4. Blocking

Take the membrane out of “Sandwich”structure, put into appropriate antibody incubation tank. Add 1 mL of Western Blocking Buffer and incubate at room temperature. For example, add 6 to 7

mL of a 6.3 × 8.3 cm membrane.

5. Primary antibody incubation

a. Dilute the primary antibody with Western Transfer Buffer at the appropriate ratio.

b. Once blocking is completed, put the diluted primary antibody working solution into the incubation tank, incubate for 2h at room temperature, or overnight at 4°C.

6. Rinse

Once primary antibody incubation is completed, rinse with TBST Buffer for 4 times, 5min each time.

7. Secondary antibody incubation

a. 10 min before completing the primary antibody washing, dilute the secondary antibody with Western Transfer at the appropriate ratio.

b. After rinsing, add the diluted secondary antibody working solution to the incubation tank, then incubate for 1h at room temperature.

8. Rinse

Once the secondary antibody incubation is done, rinse with TBST Buffer for 4 times, 5 min each time.

9. Expose

a. Based on the size of membrane, 1-2mL of working solution is required per 10cm^2 membrane. Pipette equal volumes of Solution I and SolutionII, mix well and formulate as luminescence detection working solution.

b. Take out the membrane with flat-tipped tweezers, gently touch the lower edge of the membrane

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn

with the absorbent paper to remove excess liquid from the membrane. Pipette the working solution onto the transfer membrane, ensure to a complete coverage, and incubate at room temperature for 1-2min. (This incubation can be done on clean plastic wrap or in a plastic box.)

c. Tablet press test:

Fix the membrane in the clip and press in the dark room for 1 minute develop and fix it immediately, and adjust the tableting time according to the result.

Or observe the developing and fixing by directly pressing the tablet for 30 seconds, 1, 3, 5 min, respectively.

d. Fluorescence imager detection: Put the membrane in the fluorescence imager and refer to the instrument manual for detection.

Table 1: BSA standard dilution

1mg/mL BSA(μL)	Diluent (μL)	Corresponding protein content (mg/mL)
0	20	0
1	19	0.05
2	18	0.1
4	16	0.2
8	12	0.4
12	8	0.6
16	4	0.8
20	0	1

Table 2: Concentration of the separating gel

Molecular weight (kDa)	Concentration of the separating gel (%)
X≤10	15%
10 < X≤15	13.5%
15< X≤25	12%
25< X≤35	11%
35< X≤40	10%
40< X≤55	9%
55< X≤70	8%
70< X≤100	7%
100< X	6%
Note: ” X “indicates the size of target protein	

Table 3: Preparation of the separating gel

Concentration of the separating gel (%)	6%	7%	8%	9%	10%	11%	12%	13.5 %	15%
Deionized water (mL)	5.3	4.9	4.6	4.3	4.0	3.65	3.3	2.8	2.3
30% Acrylamide (mL)	2	2.4	2.7	3.0	3.3	3.65	4.0	4.5	5.0
1.5M Tris-HCL(pH8.8) (mL)					2.5				
10%SDS (mL)					0.1				
10%AP (mL)					0.1				

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn

TEMED (mL)	0.01
Total volume (mL)	10

Table 4: Preparation of the stacking gel

	Stacking gel(5%)	Stacking gel(5%)	Stacking gel(5%)	Stacking gel(5%)	Stacking gel(5%)
Deionized water (mL)	1.1	2.1	2.7	3.4	4.1
30% Acrylamide (mL)	0.33	0.5	0.67	0.83	1.0
1.0M Tris-HCL(pH6.8) (mL)	0.25	0.38	0.5	0.63	0.75
10%SDS (mL)	0.02	0.03	0.04	0.05	0.06
10%AP (mL)	0.02	0.03	0.04	0.05	0.06
TEMED (mL)	0.002	0.003	0.004	0.005	0.006
Total volume (mL)	2	3	4	5	6

Table 5: The condition of membrane transfer

Molecular weight (kDa)	Condition of membrane transform
X≤10	Constant current200mA,30min Constant current 200mA,30min
10 < X≤15	Constant current 200mA,40min

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn

15< X≤20	Constant current 200mA,50min
20< X≤50	Constant current 200mA, 1kDa/min+20min
50< X≤100	Constant current 200mA, 1kDa/min+30min
100< X≤150	Constant current 250mA, 1kDa/min+20min
150< X≤180	Constant current270mA, 1kDa/min(Controlled time within 3h)
180< X	Constant current270mA, 1kDa/min(Controlled time within 4h)

Note: ” X “indicates the size of target protein

400-999-6126 cn.market@abclonal.com www.abclonal.com.cn