Streamlined Western Blot Complete Kit

Catalog #: RA10042

Product Overview

As a core immunological experiment, Western Blot (WB) is often a pain point for researchers due to its complex workflow and long duration (typically 1-2 days). The All-in-One Enjoy Series WB Complete Workflow Kit launched by EnkiLife includes most reagents and consumables required for the entire experiment. Users only need to prepare deionized water, ethanol/methanol, common antibodies and relevant equipment by themselves. With in-depth optimized reagents and workflow, it reduces experimental background while shortening the total experimental time to 5 hours.

Product Components

Components	Storage	10T	50T
5 × Loading buffer	-20℃	1mL	1mL*5
Calibrated Color Prestained Protein Marker (8-180kDa)	-20℃	100μL	250μL*2
Coagulant	-20℃	1ml	5ml
Lower gel 1	2-8℃	25mL	125mL
Lower gel 2	2-8℃	25mL	125mL
Upper gel 3	2-8℃	10mL	50mL
Upper gel 4	2-8℃	10mL	50mL
20 × Universal Rapid Electrophoresis Buffer	2-8℃	100mL	500mL
PVDF membrane (0.45μm)	RT	10pcs	50pcs
Filter paper-free transfer sponge	RT	6pcs	12pcs
20× Ice-Free Rapid Transfer Buffer	RT	150mL	750mL
5× Protein-free blocking diluent	2-8℃	60mL	300mL
10× TBST	RT	100mL	500mL
ECL luminescent liquid A	2-8℃	10mL	50mL
ECL luminescent liquid B	2-8℃	10mL	50mL
Manual	-	1pcs	1pcs

1. Store according to reagent requirements; shelf life is 1 year.
2. Store at 4℃; shelf life is 3 months.
3. This product should not be frozen directly. Reagents that need to be stored at -20℃ are already placed in the same resealable bag.
4. **The red component can be customized.**

Working fluid preparation

Preparation stage	Working fluid type	Preparation method
Waiting stage for rubber preparation	**Electrophoresis solution (1 L)**	20× Universal Rapid Electrophoresis Buffer :50mL ddH_2O : 950mL
Electrophoresis waiting stage	**Transfer buffer (1 L)**	20× Ice-Free Rapid Transfer Buffer: 50mL ddH_2O : 850mL Anhydrous ethanol: 100mL
	Protein-free blocking dilution (100 mL)	5× Protein-free blocking dilution: 20 mL ddH_2O : 80mL
	Washing solution (100mL)	10× TBST: 10 mL ddH_2O : 90mL

Operating procedures

1. Sample processing

Add 1/4 volume of 5× Loading buffer to the lysed sample and mix well. Heat in a boiling water bath **for 5-10 min** .

⚠Centering at high speed for 5 minutes after water bath and then collecting the supernatant can ensure sample purity and avoid precipitation affecting electrophoresis results.

2. Electrophoresis

2.1 Prepare the gel (Using a 1.0mm mini gel as an example)

2.1.1 Take 2.5 mL each of Lower gel 1 and Lower gel 2 and mix them thoroughly;

2.1.2 Add 50 μL of coagulant to the above mixture and mix gently. Pour the mixture into the gel casting plate, so that the liquid level is about 1.5 cm from the top edge of the short gel casting plate .

⚠ This solution is in excess; do not inject it all. After adding the coagulant, mix gently to avoid excessive air entering and affecting solidification .

2.1.3 Take 1.0 mL each of Upper gel 3 and Upper gel 4 (shake well before use) and mix well;

2.1.4 Add 20 μL of coagulant to the above mixture and mix gently. Pour the mixture into the gelation plate and insert the comb teeth.

2.1.5 After waiting for about **20 minutes** for the adhesive to solidify, slowly pull out the comb teeth for later use.

⚠ The preparation methods for gels of different thicknesses are as follows:

Lower layer adhesive formulation			
Gel thickness	Lower gel 1	Lower gel 2	Coagulant
0.75mm	2.0mL	2.0mL	40μL
1.0mm	2.5mL	2.5mL	50μL
1.5mm	3.8mL	3.8mL	76μL

Top layer adhesive formulation			
Gel thickness	Upper gel 3	Upper gel 4	Coagulant
0.75mm	0.8mL	0.8mL	16μL
1.0mm	1.0mL	1.0mL	20μL
1.5mm	1.5mL	1.5mL	30μL

2.2 Sample loading and electrophoresis

2.2.1 Fix the prepared gel short plates in the electrophoresis tank with the inside facing inward;

2.2.2 Add the pre-diluted electrophoresis solution to the inner tank and immerse the sample wells; the electrophoresis solution in the outer tank should appropriately cover the bottom of the gel plate ;

⚠The electrophoresis solution in the outer tank **can be used 3-4 times** .

2.2.3 Slowly add the protein marker (3-5 μL/well) and the prepared sample into the wells;

⚠ Marker is ready to use and must not be boiled.

8-180 kDa: **8**/16.5/25/31/41/52/**72**/95/130/180kDa .

10-250 kDa: 10/15/20/**25**/30/40/50/**72**/100/130/250 kDa

2.2.4 Cover the tank and connect the power supply. A constant voltage of 160-180V (≤200V) is recommended for 20-30 minutes .

⚠ The actual electrophoresis time is stopped when the bromophenol blue reaches the bottom of the gel or the markers have fully separated.

3. Transfer membrane

3.1 Remove the protective paper from both sides of the membrane, gently pick up the edge of the white PVDF membrane with clean tweezers, and immerse it in methanol solution **for 1 minute** to activate it ;

3.2 Equilibrate the gel, filter-free transfer sponge, and activated membrane in the pre-prepared

transfer solution at room temperature **for 3-5 minutes** ;
3.3 Open the transfer clamp and lay the following layers from the negative electrode (black side): filter paper-free transfer sponge → gel → PVDF membrane → filter paper-free transfer sponge. Remove any air bubbles between the gel and the membrane and lock the transfer clamp.
3.4 Insert the transfer clamp into the transfer tank, add transfer solution to completely immerse the transfer tank, and transfer at a constant current of 400mA at room temperature **for 15-30 minutes** .
⚠The transfer times for proteins of different molecular weights are as follows:

1.0mm gel		
Current	molecular weight	Transfer time
400mA	< 50kDa	10-20 min
	50-150kDa	2 0-35min
	150-200kDa	35-45min
	>200kDa	45-60min

Filter paper-free transfer sponge can be used more than 10 times after cleaning ; The transfer solution can be used 3-4 times , and the transfer time needs to be increased by 10-15 minutes for the fourth use .

4. Membrane sealing
Place the transferred PVDF membrane into 10 mL of diluted protein-free blocking buffer, place it on a shaker at room temperature for **5-10 min** , and then discard the blocking buffer .

5. Primary antibody incubation and washing
5.1 Prepare the primary antibody working solution using the diluted protein-free blocking buffer . Add 4-8 mL of the primary antibody working solution and incubate on a shaker at room temperature for **90-120 min** or overnight at 4°C.
⚠ The amount of primary antibody working solution used can be reduced appropriately after the membrane is cut .
5.2 Discard the primary antibody working solution , add 10 mL of diluted washing solution and shake to wash **for 3-5 min** . Wash 3 times, thoroughly discarding the old solution each time.
⚠ The amount of washing solution can be reduced appropriately after the membrane is cut.

6. Secondary antibody incubation and washing
6.1 Prepare the secondary antibody working solution using diluted protein-free blocking buffer . Add 4-8 mL of the secondary antibody working solution and incubate at room temperature with

shaking **for 50-60 min** .

⚠ The amount of secondary antibody working solution can be reduced appropriately after the membrane is cut .

6.2 Discard the secondary antibody working solution , add 10 mL of diluted washing solution and shake to wash for **3-5 minutes** . Wash 3 times, thoroughly discarding the old solution each time.

⚠ The amount of washing solution can be reduced appropriately after the membrane is cut.

7. Color Development and Exposure

7.1 Mix ECL luminescent solution A and ECL luminescent solution B at a ratio of 1:1 to prepare ECL working solution;

⚠ Prepare and use immediately; the total amount of membrane used is approximately 1 mL .

7.2 Use clean tweezers to remove the PVDF membrane with the protein side facing up, and evenly drop the ECL working solution onto the membrane surface and react **for 1-2 min** ;

7.3 Discard the ECL working solution and place the membrane into an imaging instrument for testing or press it into a tablet for testing.

Precautions

1. When using ECL luminescent solutions A and B, the pipette tips must be changed to prevent them from mixing and becoming ineffective.
2. The sensitivity of the ECL working solution in this kit is at the fg level;
3. Some concentrates may crystallize at 4°C ; they should be dissolved and mixed at room temperature before use.
4. To save time, reagents needed for the next experiment can be prepared while waiting for the experiment to complete. The approximate time required for each operation is as follows:

Experimental steps	Theoretical time	Estimated time
Sample processing	5-10 min	10 min
Gel preparation	20min	20min
Sample loading electrophoresis	20-30min	30min
Transfer membrane	15-30min	30min
Membrane sealing	5-10 min	12min
Primary antibody incubation and washing	100-130min	120min
Secondary antibody incubation and washing	60-75min	70min
ECL color rendering	2-5min	5min
Total time	**230-310min**	**297 min (5h)**