2-Hour Rapid Western Blot Ready-to-Use Complete Workflow Kit

Catalog No.: RA10020

Product Information

Western Blot (WB), as a core immunological assay, has long been a pain point for researchers due to its complex workflow and long duration (usually 1–2 days). The 2-Hour Rapid WB Ready-to-Use Complete Workflow Kit launched by EnkiLife contains most reagents and consumables required for a complete Western blot experiment. Users only need to prepare water, ethanol/methanol, routine antibodies and related equipment on their own.Through deeply optimized reagents and protocols, we have greatly shortened the experimental time to as fast as 110 minutes, significantly improving efficiency. Its ready-to-use design delivers a breakthrough convenient option, especially for experimental novices and researchers with tight schedules.

Experimental Step	Theoretical Duration (min)
Sample Preparation	5
Electrophoresis	25±5
Transfer (Blotting)	25±5
Blocking (Optional)	0
Primary Antibody Incubation & Washing	25±5
Secondary Antibody Incubation & Washing	25±5
ECL Development	1
Total Duration	**110±15min**

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Kit Components

Components	Storage	Size 2T (For 30 Assays)
5×Loading Buffer	-20℃	0.2mL
Calibrated Color Prestained Protein Marker (10-250kDa)	-20℃	20μL
Bis-Tris, 4-12%, 15 wells	**4℃**	2pcs
20× Universal Rapid Electrophoresis Buffer	4℃	40mL
PVDF Membrane (0.45 μm)	RT	2pcs
Filter-free Transfer Sponges	RT	4pcs
20× Ice-Bath-Free Rapid Transfer Buffer	RT	50mL
5min Protein-Free Rapid Blocking Solution	4℃	10mL
No-Blocking Rapid Antibody Diluent	4℃	40mL
Rapid Wash Buffer (10X)	4℃	20mL
ECL Substrate Solution A	4℃	1.5mL
ECL Substrate Solution B	4℃	1.5mL
Instruction Manual	-	1pcs

Note:

- Precast gels **must** be stored at 4°C, **NEVER** below 0°C.
- Store 5× Loading Buffer and Protein Marker at -20℃, and other reagents at 4℃. Shelf life: 1 year.
- If all reagents are stored at 4℃, shelf life is 3 months.
- 20× Universal Rapid Electrophoresis Buffer (outer tank buffer) and 20× Ice-Bath-Free Rapid Transfer Buffer are recommended for up to 3 uses.
- Molecular weights of the Marker: 10, 15, 20, 25, 30, 40, 50, 72, 100, 130, 250 kDa.

Operation steps

【Reagents to be prepared before experiment: 1.8 L pure water, 20 mL anhydrous methanol, 100 mL anhydrous ethanol, samples, primary antibody, secondary antibody】

1. Sample Preparation

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

⚠ Centrifuge at high speed for 5 min after heating and use the supernatant to ensure sample purity and avoid precipitate affecting electrophoresis.

2. Electrophoresis

2.1 Prepare Running Buffer（500mL）: 475 mL pure water + 25 mL 20× Universal Rapid Electrophoresis Buffer.

2.2 Assemble Precast Gel: Peel off the bottom sealing tape from the Precast Gel cassette (Bis-Tris, 4-12%, 15 wells). Slowly remove the comb. Fix the cassette into the electrophoresis tank with the short plate facing inwards.

⚠ If the U-shaped gasket has a protruding structure on top, remove it and reinstall it **upside down** so the smooth side faces outwards.

2.3 Add Running Buffer: Inner chamber: Fill completely to submerge wells. Outer chamber: Add 1/3 volume (liquid level should not exceed the gel cassette). 500 ml total for two gels.

2.4 Load Samples: Use a pipette to aspirate the Marker (included in the kit) and the samples from Step 1. Insert the pipette tip vertically and gently into the loading well, and inject the sample slowly to avoid puncturing the gel.

⚠ Recommended loading volume: 2–5 µL/well for Marker; 5–20 µL/well for samples, maximum 25 µL/well.

2.5 Run Gel: PClose the tank lid, connect to the power supply, and run at a constant voltage of 160 – 180 V (≤200 V) for **20-30 min**.

⚠ Stop electrophoresis when bromophenol blue reaches the bottom of the gel or the Marker bands are well separated. The outer tank electrophoresis buffer can be reused 3 times.

3. Transfer (Blotting)

3.1 Prepare Transfer Buffer（1L）: 50 mL 20× Ice-Bath-Free Rapid Transfer Buffer + 850 mL pure water + 100 mL anhydrous ethanol.

3.2 Activate PVDF Membrane: Peel off the light blue protective paper, and use clean tweezers to gently grasp the edge of the white PVDF membrane and immerse in methanol for **1 min** to activate.

3.3 Equilibrate Membrane & Sponges: Soak the activated membrane and Filter-free Transfer Sponges in Transfer Buffer at RT for **3-5 min**.

3.4 Equilibrate Gel: Carefully insert a gel knife/spatula into the gap between the gel plates until they separate completely. Remove the gel and equilibrate in Transfer Buffer at RT for **3-5 min**.

3.5 Assemble Transfer Sandwich: Open transfer cassette. On the **cathode (black side)**, stack in order: Sponge → Gel → PVDF Membrane → Sponge. Close and lock the cassette.

⚠ **Thoroughly remove all bubbles** between the gel and membrane.

3.6 Transfer: Place cassette into transfer tank filled with Transfer Buffer (completely submerged). Transfer at 400 mA constant current, RT, for **25-35 min**.

3.7 Wash the Membrane: Remove the transferred PVDF membrane, rinse once with pure water (approx. 50 mL) to prevent drying, and recover the transfer buffer (reusable 3 times).

4. Membrane Blocking(Optional; not required for first-use primary antibody)

Immerse the rinsed PVDF membrane in 5–10 mL protein-free rapid blocking solution, and block on a shaker at room temperature for **5–7 min**. Discard the blocking solution.

5. Primary Antibody Incubation

5.1 Prepare Primary Antibody Working Solution: Dilute the primary antibody with No-Blocking Rapid Antibody Diluent according to the ratio specified in the primary antibody datasheet. (EnkiLife offers tens of thousands of high-quality primary antibodies).

5.2 Incubate Primary Antibody: Add 5–10 mL primary antibody working solution to submerge the PVDF membrane, and incubate on a shaker at RT for **20–30 min**.

⚠ Alternative incubation: 90 minutes or overnight at 4℃. Cutting the membrane reduces the required volume of primary antibody working solution; blocking is required when incubating with recovered primary antibody.

6. Primary Antibody Wash

6.1 Prepare Wash Buffer(100mL): 10 mL rapid wash buffer (10×) + 90 mL pure water, mix well.

6.2 Wash: Discard primary antibody solution. Add 10 ml Wash Buffer and wash on shaker for **0.5 min**. Repeat 3 times, **discarding the wash solution completely** each time.

⚠ The primary antibody can be recovered and stored at 4℃ for reuse. Blocking is required when incubating with recovered primary antibody.

7. Secondary Antibody Incubation

7.1 Prepare Secondary Antibody Working Solution: Dilute the secondary antibody according to its datasheet using No-Blocking Rapid Antibody Diluent. (EnkiLife offers high-quality secondary antibodies for multiple species).

7.2 Incubate with Secondary Antibody: Discard the final wash solution. Add 5-10 mL of Secondary Antibody Working Solution. Incubate on a shaker at RT for **20-30 min**.

⚠ Alternative incubation: ⩾60 minutes at 4℃. Reduce the volume of secondary antibody working solution appropriately for cut membranes.

8. Secondary Antibody Wash

Discard secondary antibody solution. Add 10 ml Wash Buffer and wash on shaker for **0.5 min**. Repeat 3 times, **discarding the wash solution completely** each time.

⚠ The secondary antibody can be recovered and stored at 4℃ for reuse..

9. Detection (ECL)

9.1 Prepare ECL Working Solution (1mL): Mix 0.5 ml ECL Solution A and 0.5 ml ECL Solution B to prepare 1 ml ECL Working Solution.

⚠ Approx. 0.5–1 mL is needed for a whole membrane; reduce volume appropriately for cut.

9.2 Detection:

9.2.1 Remove the PVDF membrane with clean tweezers, drain excess liquid (keep moist), and lay flat in the incubation box (protein side facing up).

9.2.2 Drop the ECL working solution evenly onto the PVDF membrane and incubate for **1–2 min**.

9.2.3 Aspirate excess ECL working solution, place the PVDF membrane on the luminescence platform, and detect with a chemiluminescence imager.

Precautions

1. Precast gels must be stored at 4°C. Do not store below 0°C.
2. Blocking is not required for this kit. However, if the primary antibody is not diluted with blocking-free rapid antibody diluent, or if the pre-diluted primary antibody has been used before, the membrane must be blocked prior to primary antibody incubation.

3. To save time, reagents for the next step can be prepared during waiting periods of the experiment.
4. Both electrophoresis buffer and transfer buffer can be reused 3 times, but fresh electrophoresis buffer must be used for the inner tank.
5. Diluted electrophoresis buffer, transfer buffer and wash buffer can be stored for more than 1 month.
6. Keep the protein side facing up for all subsequent operations on the transferred membrane (except when equipment specifies otherwise).
7. Appropriately extending antibody incubation and washing time can further improve band clarity and background cleanliness.
8. Always use a fresh pipette tip for ECL Luminescence Reagent A and B to avoid cross-contamination and reagent inactivation.