4-Hour Fast Western Blot Ready-to-Use Complete Workflow Kit

Catalog No.: RA10021

Product Information

Western Blot (WB), a core immunological technique, is often a pain point for researchers due to its complex workflow and lengthy duration (typically 1-2 days). EnkiLife's newly launched 2-Hour Rapid Western Blot Ready-to-Use Complete Workflow Kit provides a revolutionary solution. This kit contains the vast majority of reagents and consumables required for the entire experiment; users only need to supply water, ethanol/methanol, common antibodies, and related equipment. Through deeply optimized reagents and processes, we have dramatically reduced the total experiment time to as little as 2 hours, significantly enhancing efficiency. This breakthrough offers unprecedented convenience, especially for beginners and researchers under tight deadlines.

Components

Components	Storage	Size 2T(For 30 Assays)
Protein Loading Buffer (5X)	-20℃	0.2ml
Pre-stained Protein Marker (10-180 kDa)	-20℃	20ul
Precast Gel (Bis-Tris, 4-20%, 15 wells)	**4℃**	2pcs
Electrophoresis Buffer Granules	RT	2pcs (500ml/pcs)
PVDF Membrane (0.45 μm)	RT	2pcs
Filter-free Transfer Sponges	RT	4pcs
Rapid Transfer Buffer Granules	RT	2pcs (1L/pcs)
Protein-Free Rapid Blocking Buffer	4℃	20ml
Antibody Diluent	4℃	20ml
Wash Buffer (10X)	4℃	14ml
ECL Substrate Solution A	4℃	1.2ml
ECL Substrate Solution B	4℃	1.2ml

Shelf Life:

12 months: When stored per component requirements.

3 months: When all components stored at 4°C.

Note:

Precast gels **must** be stored at 4°C, **NEVER** below 0°C.

Operation steps

1. **Sample Preparation**

Add 1/4 volume of Protein Loading Buffer (5X) 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: Add ~400 ml pure water to 1 pack of Electrophoresis Buffer Granules. Stir to dissolve. Bring volume to 500 ml with pure water.

2.2 Assemble Precast Gel: Peel off the bottom sealing tape from the Precast Gel cassette (Bis-Tris, 4-20%, 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: Load samples from step 1. Insert pipette tip vertically into the well and dispense sample slowly to avoid puncturing the gel.

⚠ Recommended loading volume: 5-20 µl/well, max 25 µl/well.

2.5 Add Marker: Load Pre-stained Marker (10-180 kDa) as above. Recommended: 2-5 µl/well.

⚠ Marker is ready-to-use. **Do not boil**. Molecular weights: 10/15/25/35/40/55/70/100/130/180 kDa.

2.6 Run Gel: Place lid on tank, connect power supply. Run at 140-150 V (≤180 V) for **40-50 min**.

⚠ Actual run time: Stop when the bromophenol blue dye front reaches the bottom of the gel or Pre-stained Marker bands are adequately separated.

3. Transfer (Blotting)

3.1 Prepare Transfer Buffer: Add 1 pack of Rapid Transfer Buffer Granules to 800 ml pure water and mix well. Add 100 ml absolute ethanol/methanol and mix well. Bring volume to 1 L with pure water.

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 **20-30 min**.

4. Membrane Blocking

Place the transferred PVDF membrane into an incubation box containing 10 ml Protein-Free Rapid Blocking Buffer. Incubate on a shaker at RT for **10 min**.

5. Primary Antibody Incubation

5.1 Prepare Primary Antibody Working Solution: Dilute the primary antibody according to its datasheet using Rapid Antibody Diluent. (EnkiLife offers thousands of high-quality primary antibodies).

5.2 Incubate with Primary Antibody: Discard the blocking solution. Add 5 ml of Primary Antibody Working Solution. Incubate on a shaker at RT for **60-90 min** or 4°C overnight.

⚠ Reduce volume for cut membranes.

6. Primary Antibody Wash

6.1 Prepare Wash Buffer: Mix 1 volume Rapid Wash Buffer (10X) with 9 volumes pure water.

6.2 Wash: Discard primary antibody solution. Add 10 ml Wash Buffer and wash on shaker for **5 min**. Repeat 3 times, **discarding the wash solution completely** each time.

⚠ Primary antibody can be recovered for reuse. For cut membranes, reduce wash buffer volume appropriately.

7. Secondary Antibody Incubation

7.1 Prepare Secondary Antibody Working Solution: Dilute the secondary antibody according to its datasheet using 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 ml of Secondary Antibody Working Solution. Incubate on a shaker at RT for **40-60 min** or 4°C overnight.

⚠ Reduce volume for cut membranes.

8. Secondary Antibody Wash

Discard secondary antibody solution. Add 10 ml Wash Buffer and wash on shaker for **5 min**. Repeat 3 times, **discarding the wash solution completely** each time.

⚠ Secondary antibody can be recovered for reuse. For cut membranes, reduce wash buffer volume appropriately.

9. Detection (ECL)

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

⚠ Prepare fresh. Use ~0.1 ml/cm^2 membrane (approx. 1 ml for a full membrane).

9.2 Detection:

9.2.1 Using clean forceps, remove PVDF membrane. Drain excess liquid (keep moist). Lay membrane protein-side up on plastic wrap.

9.2.2 Apply ECL Working Solution evenly over the membrane surface. Incubate for **1-2 min**.

9.2.3 Drain off excess ECL Working Solution. Sandwich the membrane between two sheets of plastic wrap.

9.2.4 Proceed to detection using a chemiluminescence imager or X-ray film.

⚠ Avoid bubbles or wrinkles between the membrane and plastic wrap.

Notes

1. **Precast gels must be stored at 4°C. Do not store below 0°C.**

2. **Always use separate pipette tips when dispensing ECL Solution A and Solution B** to prevent cross-contamination and reagent inactivation.
3. To save time, prepare reagents for the next step during incubation/wait periods.
4. The ECL Working Solution in this kit offers **fg-level sensitivity**.