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

Catalog No.: RA10021

Product Information

Western Blot (WB), as a core immunological experiment, often becomes a pain point for researchers due to its complex process and long time consumption (usually 1-2 days). EnkiLife's 4-Hour Fast Western Blot Ready-to-Use Complete Workflow Kit provides you with a revolutionary solution. This kit contains the majority of reagents and consumables required for a complete experiment. Users only need to bring their own water, ethanol/methanol, commonly used antibodies, and related equipment. Through deep optimization of reagents and processes, we have shortened the experimental time to as fast as 3.6 hours, significantly improving efficiency. The ready to use design provides a breakthrough and convenient choice for novice experimenters and time sensitive researchers.

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**.