

ab194946 – Western Blot Protocol for FAM26F (Transmembrane Protein)

The following Western Blot procedure has been evaluated for successful detection of FAM26F (Transmembrane Protein).

For overview, typical data and additional information please visit: www.abcam.com/ab194946

These materials and reagents will be required to successfully utilize this procedure:

- Lysis Buffer
- PBS
- SDS sample buffer
- 10–12% resolving gel
- Tank transfer system
- PVDF membrane
- Methanol
- Ponceau S
- BSA
- TBS-T
- HRP-conjugated anti-rabbit IgG
- Loading controls
- ECL substrate
- Chemiluminescence imaging system

1. Cell Lysis & Protein Extraction

Lysis Buffer:

- 50 mM Tris-HCl (pH 7.4)
- 150 mM NaCl
- 1% Triton X-100 or 0.5% NP-40
- 0.1% SDS
- 0.5% sodium deoxycholate (optional)
- Protease/phosphatase inhibitors

Procedure:

1. Wash cells/tissue with ice-cold PBS.
2. Add cold lysis buffer (1 mL per 10⁶ cells).
3. Incubate on ice for 30 minutes with occasional mixing.
4. Centrifuge at 14,000 × g for 15 minutes at 4°C.
5. Collect supernatant and measure protein concentration (e.g., BCA assay).

2. Sample Preparation

- Mix lysate with 4× SDS sample buffer (final 1×).
- Heat at **70°C for 10 minutes** (avoid 95°C).
- Spin briefly to remove aggregates.

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3. SDS-PAGE Electrophoresis

- Use 10–12% resolving gel.
- Load 20–40 µg of total protein per lane.
- Run gel at 80 V (stacking), then 120 V (resolving).

4. Protein Transfer

- Use PVDF membrane (pre-wet with methanol).
- Wet transfer recommended for hydrophobic proteins.
- Transfer at 100 V for 1–1.5 hours at 4°C in buffer with 10% methanol ± 0.05% SDS.
- Stain with Ponceau S to confirm transfer.

5. Blocking

- Block in **5% BSA in TBS-T** for 1 hour at room temperature.

6. Antibody Incubation

Primary Antibody:

- Anti-FAM26F (e.g., rabbit polyclonal)
- Dilution: 1:500 to 1:1000 in 5% BSA/TBS-T
- Incubate overnight at 4°C

Secondary Antibody:

- HRP-conjugated anti-rabbit IgG
- Dilution: 1:5000–1:10000
- Incubate 1 hour at room temperature

Note: A loading control such as Na⁺/K⁺-ATPase, Calnexin, or GAPDH should be used.

7. Detection

- Wash 3 × 5 min with TBS-T
- Add ECL substrate
- Detect using chemiluminescence imaging system

Optional Notes

- IFN-γ treatment can upregulate FAM26F expression.
- For difficult-to-solubilize samples, consider using digitonin or n-dodecyl-β-D-maltoside.

Technical Support

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