YamayBio

# Western Blot
# Frequently Asked Questions

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# 1. General Recommendations

- We recommend first **quantifying** the protein concentration using a BCA assay (BH5484) or Bradford assay (BH5485), and then normalizing the loading amount for each sample based on the quantification results.
    - For routine WB experiments: **20–30 µg/lane** is recommended.
    - For highly abundant proteins: **5–15 µg/lane** may be sufficient.
    - For low-abundance proteins: **30–50 µg/lane** can be considered.
- Avoid repeated freeze-thaw cycles of protein samples
- Use fresh buffers and reagents whenever possible
- Verify compatibility between antibodies, membranes, and substrates
- Handle gels and membranes carefully to avoid damage
- Include positive and negative controls when troubleshooting experiments

# 2. Why do I see multiple bands at different molecular weights?

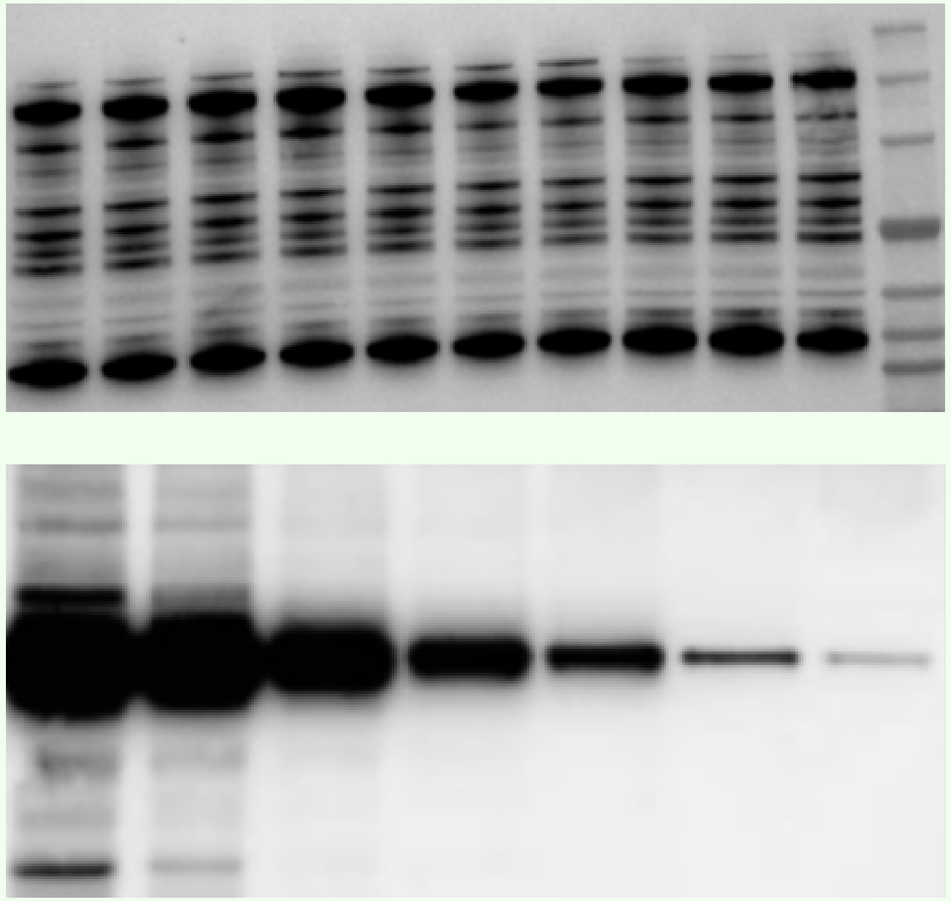
## Possible Causes

1. Protein degradation by proteases
2. Post-translational modifications (e.g. ubiquitination)
3. Poor antibody specificity
4. Protein multimer formation
5. Protein cleavage products
6. Excessive protein loading

## Suggested Solutions

- Use fresh samples and protease inhibitors
- Review literature regarding protein conformations and modifications
- Verify antibody specificity and optimize antibody dilution
- Quantify protein concentration and reduce protein loading amount if necessary

**Representative Western Blot Patterns**



# 3.Why does my blot have a high background signal?

## Possible Causes

1. Antibody interaction with blocking reagent
2. Post-translational modifications (e.g. ubiquitination)
3. Insufficient washing
4. Inadequate blocking
5. Contaminated buffers

## Suggested Solutions

- Use a different blocking reagent
- Reduce antibody concentration
- Increase washing duration and frequency
- Optimize blocking conditions
- Prepare fresh buffers

### Representative Western Blot Patterns

# 4.Why are there black spots on my membrane?

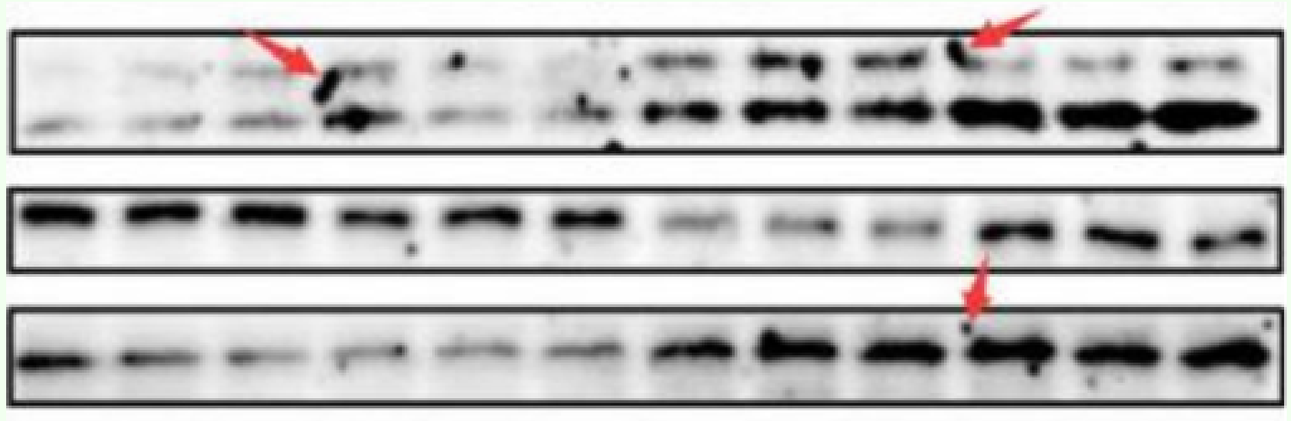
## Possible Causes

1. Undissolved milk particles in blocking solution
2. Membrane contamination

## Suggested Solutions

- Ensure blocking powder is completely dissolved
- Use fresh membranes
- Use a protein free blocking buffer (BM3152)

### Representative Western Blot Patterns



# 5.Why are my bands weak or absent?

## Possible Causes

1. Low target protein expression
2. Protein degradation
3. Insufficient protein loading
4. Viscous protein samples impairing migration
5. Incomplete transfer
6. Over-transfer
7. Incorrect primary/secondary antibody pairing
8. Low antibody concentration
9. Poor antibody performance
10. Low ECL substrate sensitivity

## Suggested Solutions

- Use fresh samples and protease inhibitors
- Increase protein loading amount
- Reduce sample viscosity or protein concentration
- Confirm transfer efficiency with membrane and/or gel staining
- Optimize transfer conditions
- Verify antibody compatibility and specificity
- Optimize antibody dilution
- Use a higher-sensitivity substrate
- Increase exposure time

**Representative Western Blot Patterns**

# 6.Why do my bands not match the predicted molecular weight?

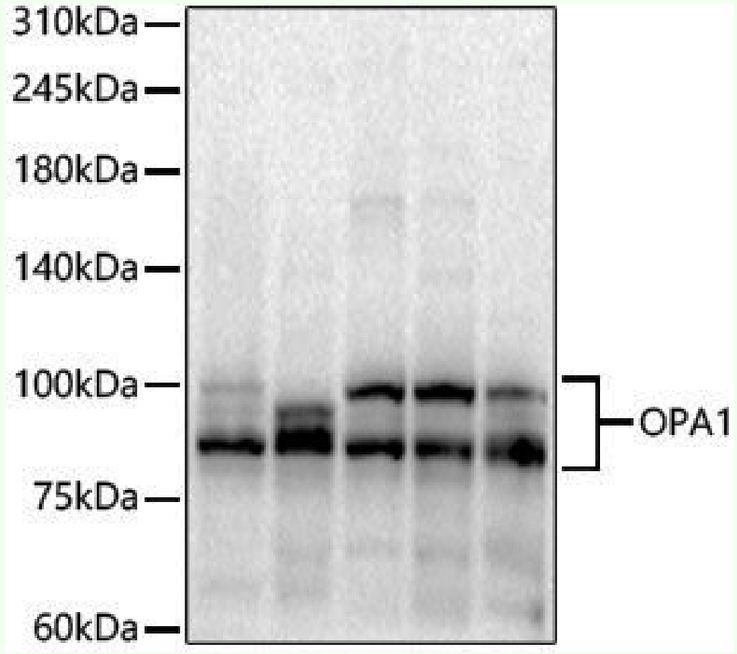
## Possible Causes

1. Post-translational modifications
2. Protein aggregation or multimer formation
3. Protein degradation
4. Incorrect molecular weight marker calibration

## Suggested Solutions

- Verify phosphorylation or glycosylation events
- Check for aggregation under non-reducing conditions
- Reduce sample viscosity or protein concentration
- Confirm transfer efficiency with membrane and/or gel staining

### Representative Western Blot Patterns




OPA1 is normally detected as two bands: **L-OPA1** at approximately **100 kDa** and **S-OPA1** at approximately **80–90 kDa**. If only an ~80 kDa band is observed, L-OPA1 may have been completely **cleaved** into S-OPA1.

# 7.Why are my protein bands distorted or crooked?

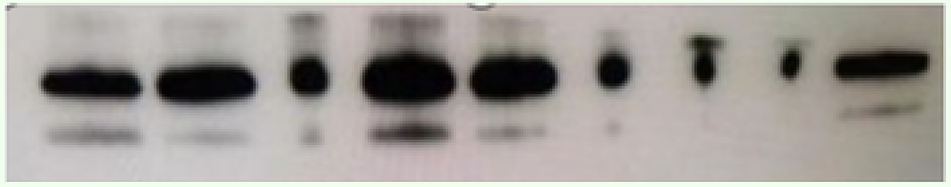
## Possible Causes

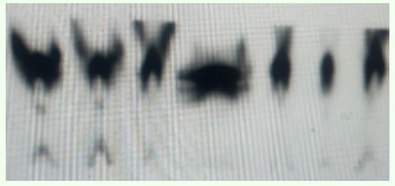
1. Excessive protein loading
2. High sample viscosity
3. Uneven gel polymerization
4. Improper gel insertion
5. Uneven electrophoresis conditions
6. Air bubbles during transfer
7. Damaged gel or membrane
8. Excessive heat during electrophoresis

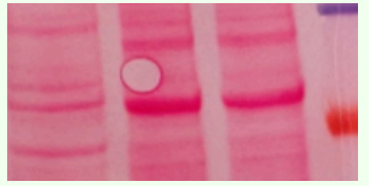
## Suggested Solutions

- Reduce protein loading amount
- Centrifuge or sonicate viscous samples
- Dilute samples with loading buffer
- Ensure even gel casting
- Confirm proper gel positioning
- Remove all air bubbles during transfer assembly
- Use fresh running buffer
- Avoid overheating during electrophoresis

**Representative Western Blot Patterns**







These examples are likely caused by issues with sample preparation.

# 8.Why are my protein bands smeared?

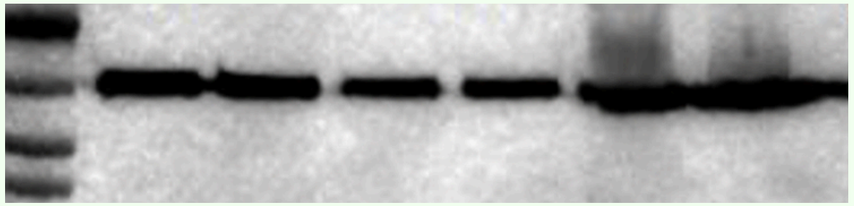
## Possible Causes

1. Protein overload
2. Sample degradation
3. Salt contamination
4. Incomplete sample denaturation
5. Running voltage too high

## Suggested Solutions

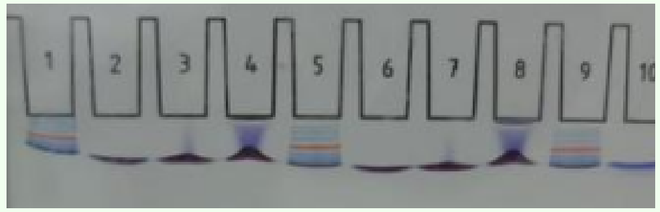
- Reduce protein loading amount
- Prepare fresh samples
- Reduce salt concentration
- Fully denature samples before loading
- Run gels at recommended voltage

### Representative Western Blot Patterns



Upward Smearing

Downward Smearing




Loading Well Smearing

# 9.Why is my transfer efficiency poor?

| Possible Causes | Suggested Solutions |
| --- | --- |
| 1 Improper transfer conditions | Optimize transfer time and voltage/current according to protein size; increase transfer time for large proteins and avoid over-transfer for small proteins. |
| 2 Improper gel or membrane type | Use an appropriate gel percentage and membrane pore size; for large proteins, consider lower-percentage gels and optimize methanol/SDS conditions. |
| 3 Incorrect transfer buffer composition | Prepare fresh transfer buffer; adjust methanol concentration and add a small amount of SDS when needed for high-MW proteins. |
| 4 Incorrect transfer assembly | Remove all air bubbles, ensure full contact between gel and membrane, and confirm the correct transfer orientation before running. |
| 5 Excessive heat during transfer | Keep the transfer system cool using an ice pack or cooling system; reduce voltage/current if excessive heating occurs. |

Still can’t find an answer?

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