SDS-PAGE and Western Blot Protocol

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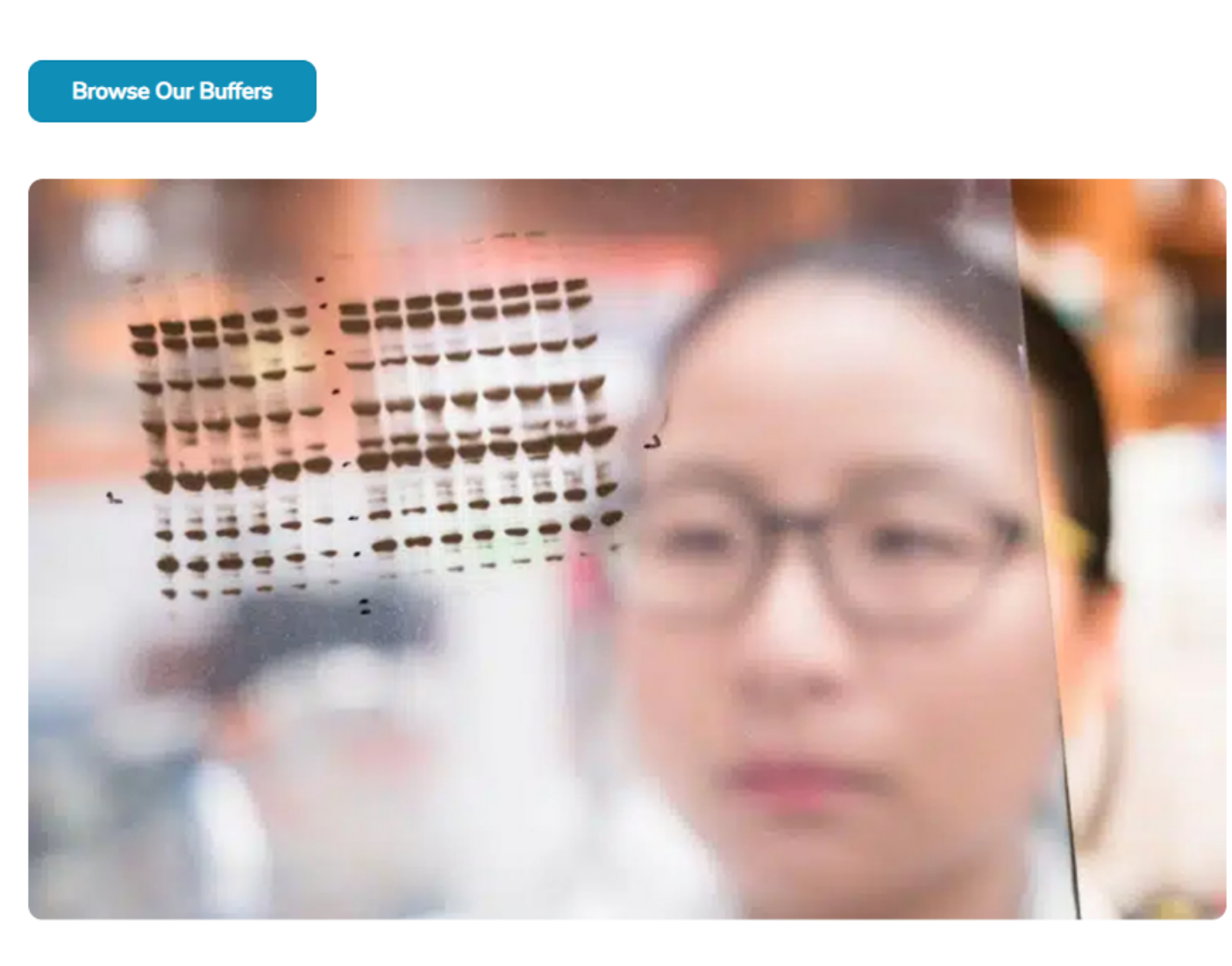
Introduction

SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) and Western blotting are complementary techniques used to analyze proteins. While SDS-PAGE is a fundamental technique for separating proteins by molecular weight, western blotting allows for the transfer of these proteins onto a membrane, enabling specific identification. Together, these techniques provide a powerful approach for studying protein expression, structure, and function.

However, issues such as poor protein transfer, weak signals, or high background noise can compromise data quality, hindering the reliability and reproducibility of results. Using high-quality reagents, such as those supplied by Boston BioProducts, alongside an optimized protocol can minimize the risk of these common challenges.

Our SDS-PAGE and Western blot protocol offers detailed, step-by-step instructions, troubleshooting tips, and our best practices to help you obtain reliable, high-quality data.

Browse Our Buffers



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Need to keep our SDS-PAGE and western blot protocol on hand for quick reference in the lab?

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Further Reading

See how our SDS-PAGE and western blotting reagents are making a splash in the world of research.

Optimized gene transduction in human lung organoids: A high-efficiency method for advanced research applications (Khateeb, Liang, Li et al., 2025)

Subtype-specific role for Jagged1 in promoting or inhibiting breast tumor formation (Chung, Wang, Challagundla et al., 2025)

Materials & Reagents

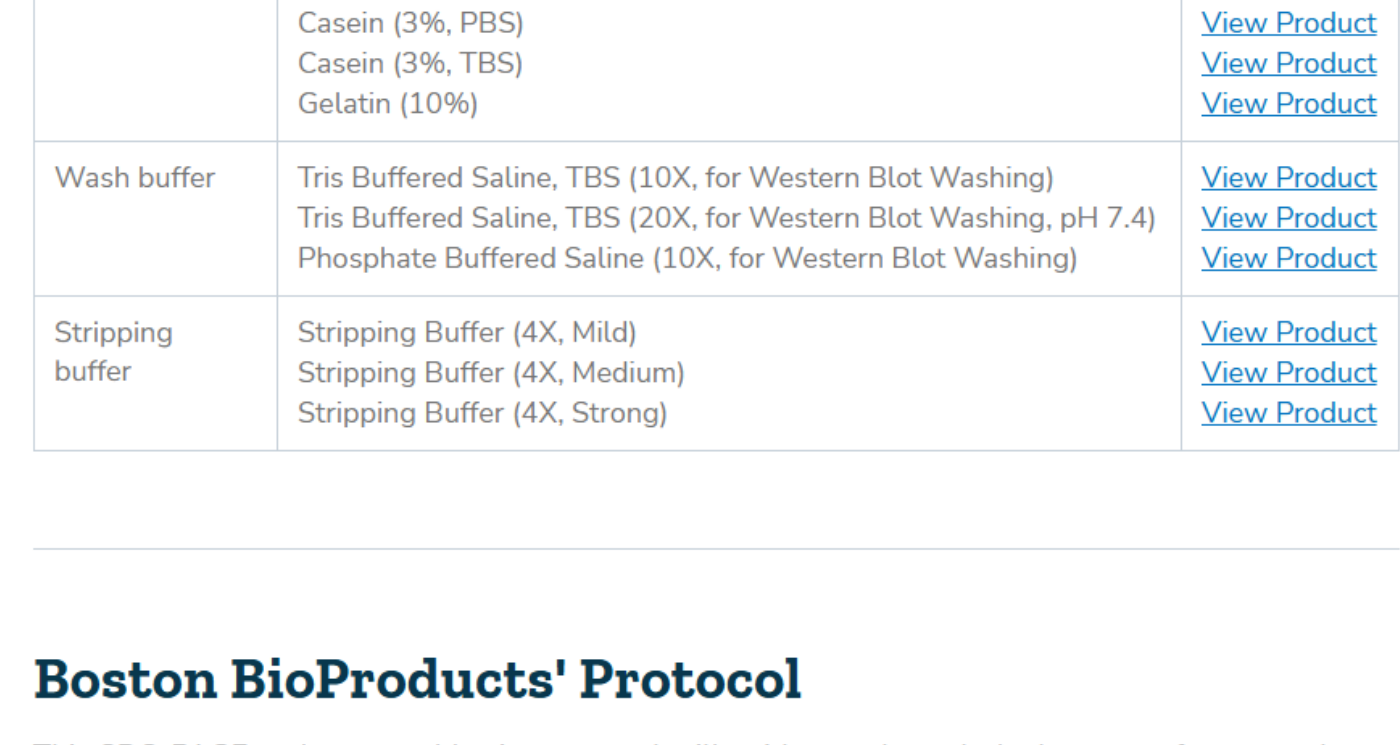
Here's everything you need to carry out our SDS-PAGE and western blot protocol. In addition to Boston BioProducts' high-quality buffers and reagents, you'll need the following materials and equipment:

- Pipettes and tips
- Microcentrifuge tubes
- Heat block (or water bath)
- Electrophoresis chamber and power supply
- Western blot transfer system
- Membrane (PVDF or nitrocellulose)
- Antibodies (primary and secondary)
- Imaging system

Reagent	Product Name	Link
Lysis buffer	RIPA buffer NP-40 lysis buffer Triton X-100 Lysis Buffer CHAPS Lysis Buffer	View Product View Product View Product View Product
Laemmli buffer	Laemmli SDS-Sample Buffer (6X, Non-Reducing) Laemmli SDS-Sample Buffer (6X, Reducing) Laemmli SDS-Sample Buffer (4X, Non-Reducing) Laemmli SDS-Sample Buffer (4X, Reducing)	View Product View Product View Product View Product
SDS solution	Sodium Deoxycholate Solution (10%) Sodium Dodecyl Sulfate, SDS (20%) Sodium Dodecyl Sulfate, SDS (10%)	View Product View Product View Product
Acrylamide Solution	Acrylamide (30%) Acrylamide (40%) Bis-Acrylamide BioAcryl-P (30%, 29:1) BioAcryl-P (40%, 29:1) BioAcryl-P (30%, 37.5:1) BioAcryl-P (40%, 37.5:1) BioAcryl-P (30%, 39:1) BioAcryl-P (40%, 39:1)	View Product View Product View Product View Product View Product View Product View Product View Product View Product
Stacking buffer	Stacking buffer	View Product
Separating buffer	Separating (Resolving) Buffer	View Product
Deionized water	Reverse Osmosis Deionized Water	View Product
Running buffer	Tris-Glycine-Native Running Buffer (10X) Tris-Glycine-SDS Running Buffer (10X) MOPS-SDS Running Buffer (20X) MES-SDS Buffer (20X)	View Product View Product View Product View Product
Transfer buffer	Transfer Buffer (10X, Electro Blotting) Semi-Dry Blot Transfer Buffer (10X) Bis-Tris Transfer Buffer (20X)	View Product View Product View Product
Ponceau	Ponceau S Solution	View Product
Blocking buffer	Non-Fat Powdered Milk (Blotto) Bovine Serum Albumin, BSA (5%, TBST) Bovine Serum Albumin, BSA (5%, TBS) Bovine Serum Albumin, BSA (3%, PBS) Bovine Serum Albumin, BSA (3%, TBS) Bovine Serum Albumin, BSA (3%, TBST) Bovine Serum Albumin, BSA (3% in PBST) Casein (3%, PBS) Casein (3%, TBS) Gelatin (10%)	View Product View Product View Product View Product View Product View Product View Product View Product View Product View Product
Wash buffer	Tris Buffered Saline, TBS (10X, for Western Blot Washing) Tris Buffered Saline, TBS (20X, for Western Blot Washing, pH 7.4) Phosphate Buffered Saline (10X, for Western Blot Washing)	View Product View Product View Product
Stripping buffer	Stripping Buffer (4X, Mild) Stripping Buffer (4X, Medium) Stripping Buffer (4X, Strong)	View Product View Product View Product

Boston BioProducts' Protocol

This SDS-PAGE and western blotting protocol will guide you through the key steps from sample preparation to detection, to help ensure clear and reproducible data and western blotting success!



Sample Preparation

Proper sample preparation is critical for obtaining reliable and reproducible Western blot results. This step ensures that proteins are extracted efficiently, remain intact, and are accurately quantified before SDS-PAGE. Below, we outline the key steps to prepare your samples for analysis.

1. Collect and label your samples carefully, and dilute them as necessary.

   💡 Remember to keep your samples on ice to prevent degradation!

2. Choose a lysis buffer based on your sample type and desired downstream applications (check out our recommendations). Add it to your samples to lyse them.

   ⚠ Remember to add protease inhibitors.

   💡 Choose the appropriate lysis buffer: NP-40 for whole cell lysate; RIPA membrane-bound, nuclear, or mitochondrial proteins; Tris-HCL for cytoplasmic proteins.

3. Centrifuge your samples to remove insoluble debris (we use 14,000 x g for 15 minutes for cell samples).

   Then, transfer the supernatant to a clean microcentrifuge tube and discard the pellet.

4. Measure the protein concentration of your lysate using an appropriate assay, such as Bradford or BCA, to ensure equal loading across samples.

   ⚠ Use BCA assay if working with samples that contain <5% detergent for higher sensitivity.

5. Prepare your protein samples for SDS-PAGE by taking the recommended protein amount (10-50µg/lane) and diluting it in a sample buffer such as Laemmli. Heat the samples at 95°C for 5 minutes to fully denature the proteins.

SDS-PAGE

SDS-PAGE is a widely used technique to separate proteins based on molecular weight. The process involves denaturing proteins with SDS, which imparts a uniform negative charge, allowing proteins to migrate through a polyacrylamide gel under an electric field. The following steps guide you through gel preparation, sample loading, and electrophoresis conditions to achieve optimal protein separation.

1. Prepare the resolving and stacking gels. The table below provides a basic recipe to make 1 gel (10% acrylamide). Make sure you optimize it based on the size of your protein of interest.

   💡 Larger proteins should have a lower percentage of acrylamide in the gel.

   ⚠ Remember to create the wells in your gel by carefully inserting a comb while the stacking gel is still wet.

Component	Separating gel (10%)	Stacking gel (4%)
Acrylamide (40%)	1.25mL	0.25mL
Separating buffer (4X)	1.25mL	-
Stacking buffer (4X)	-	0.625mL
Deionized water	2.5mL	1.625mL
10% APS	50µL	25µL
TEMED	5µL	2.5µL
Total (mL)	5mL	2.5mL

   ⚠ Only add the APS and TEMED when you are ready for the gel to set!

2. Once the gel is set, gently remove the comb. It's time to load equally across wells. Include a molecular weight ladder in one of the lanes.

   ⚠ Be careful not to touch the bottom of the wells with your pipette tip, as this can create distorted bands.

   💡 It helps to load your samples asymmetrically to help orient the gel during transfer.

3. Run the gel at a constant voltage (100-200V) until the desired separation is achieved.

   ⚠ Running times and voltages should be optimized, e.g., larger proteins will require a higher voltage for a longer period of time.

4. Once SDS-PAGE is complete, carefully pry open the apparatus with a gel knife and remove the gel.

   💡 Use an ice pack or perform the transfer in a cold room to prevent overheating.

Protein Transfer

Following electrophoresis, proteins must be transferred from the gel onto a membrane for detection. This transfer step is crucial to ensure that target proteins are accessible for antibody binding. There are two primary transfer methods: wet transfer, which is ideal for larger proteins, and semi-dry transfer, which is faster and suited for smaller proteins. Below, we outline the steps for each method and tips to ensure efficient protein transfer.

1. Choose your membrane type:
   - PVDF has a higher binding capacity, so is best for low-expressed proteins.
   - Nitrocellulose is ideal for proteins with low molecular weight.

   💡 For a fluorescent western blot, Ponceau staining is not recommended as it can cause high background fluorescence, even after washing.

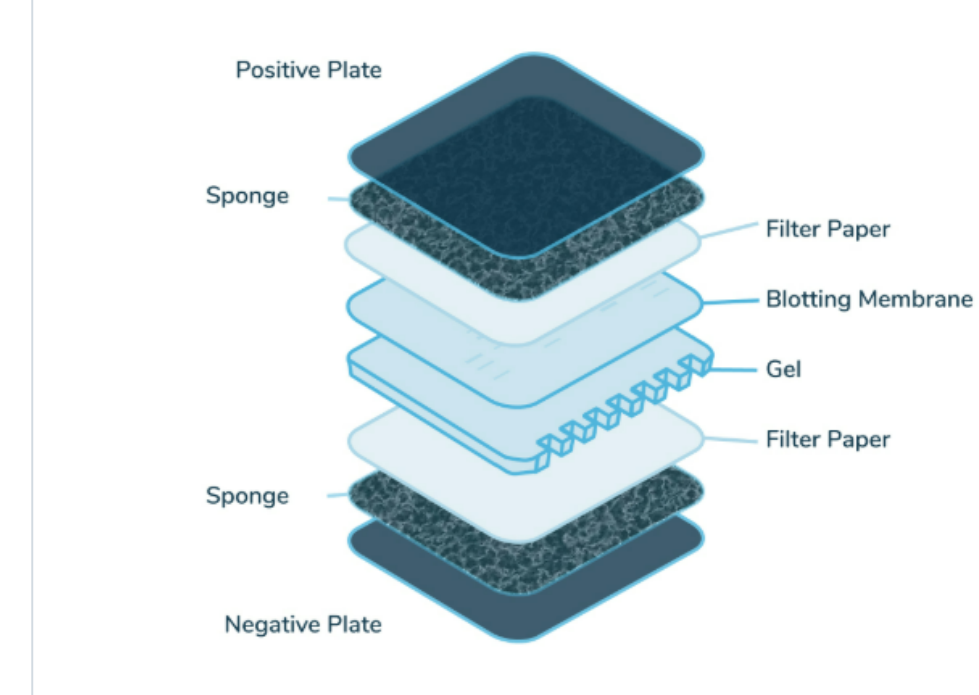
   ⚠ If you are using PVDF, you must first soak the membrane in methanol for 30 seconds.

2. Soak the membrane in transfer buffer for 10 minutes to help equilibrate the gel.

3. Prepare the transfer sandwich in the transfer cassette according to the illustration below.

   ⚠ Make sure you gently remove air bubbles with a small roller or pipette.

   💡 Make sure you put the gel closest to the negative electrode and the membrane closest to the positive electrode.



4. Perform either a semi-dry or wet transfer.

   💡 Choose semi-dry transfer for a faster, easier transfer of small-volume or small-sized (<30kDa) proteins. Opt for wet transfer if you want to transfer a larger (>100kDa) target protein.

   Semi-Dry Transfer:
   - For this method, place the transfer sandwich horizontally between the two plate electrodes in the transfer apparatus.
   - Apply a constant current (0.1 to 0.4 A) or voltage (10 to 24 V) for 10 minutes, or up to an hour.

   Wet Transfer:
   - For this method, cushion the transfer sandwich with pads, press them together with a support grid, and place them vertically in a tank filled with transfer buffer.
   - Apply a constant current (30V or 1A) for one hour, or up to overnight.

5. (Optional) Confirm the success of the protein transfer using Ponceau.

Blocking and Antibody Incubation

Blocking and antibody incubation are key steps in Western blotting, ensuring specific binding of antibodies to the target protein while minimizing background noise. The choice of blocking buffer, antibody concentration, and incubation times can significantly impact the clarity and specificity of your results. Follow the steps below to optimize your antibody binding for high-quality detection.

1. Incubate the membrane in a blocking buffer for either one hour at room temperature, or overnight at 4°C with gentle rocking.

   💡 The blocking buffer can be either BSA, milk powder, casein, or Gelatin. For guidance on which to choose, see our FAQs.

2. Dilute the primary antibody to working concentration with blocking buffer and incubate the membrane in the solution (either with gentle rocking overnight at 4 °C, or 1 hour at room temperature).
3. Wash the membrane with wash buffer (either TBS, TBST, or PBS) for 10 minutes, three times.
4. Incubate the membrane with the diluted secondary antibody for one hour at room temperature.
5. Wash the membrane again (for 10 minutes, three times) to remove any unbound antibody.

Detection and Quantification

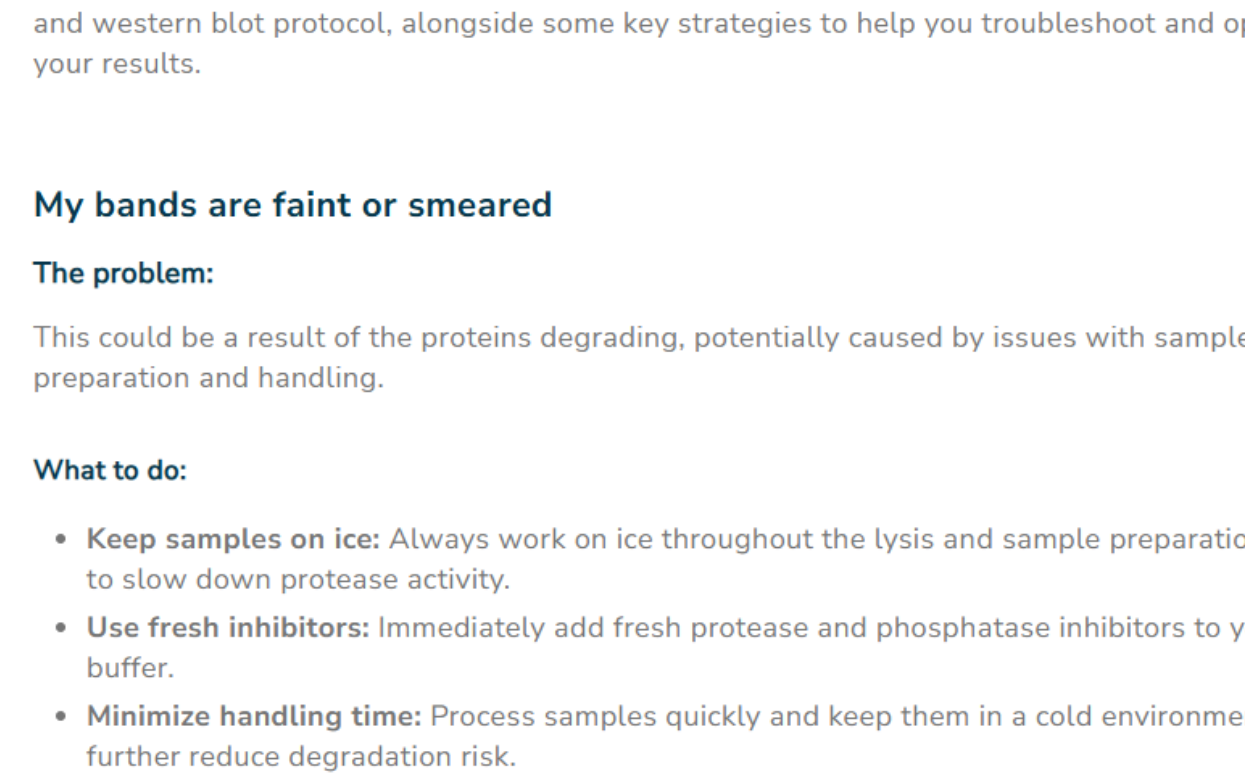
Once antibodies have bound to their target proteins, detection methods such as chemiluminescence, fluorescence, or colorimetric assays are used to visualize protein expression. Proper imaging and quantification help ensure that results are reproducible and provide meaningful biological insights. Below, we outline the key steps for detecting and analyzing your protein bands.

1. If using or chemiluminescent detection method, prepare the chemiluminescent substrate solution according to the manufacturer's instructions and incubate the blot in the substrate solution for 1-5 minutes.

   ⚠ Remove any excess substrate by dabbing the edge of the blot with tissue paper.

2. Place the blot in the imaging system tray or scanner.
3. Image your blot, adjusting exposure as needed.
4. Remove the membranes from the scan bed, and clean the image scanning bed.
5. To quantify, utilize image analysis software that supports band detection and quantification.

Troubleshooting & Optimization Tips for SDS-PAGE and Western Blot Protocols



Even the most well-planned experiments can encounter unexpected challenges. Common issues such as poor protein transfer, weak signals, and high background noise can significantly impact data quality and reproducibility.

Using high-quality buffers and reagents, combined with a well-optimized protocol, can minimize the risk of these common challenges. Below are some common issues encountered during the SDS page and western blot protocol, alongside some key strategies to help you troubleshoot and optimize your results.

My bands are faint or smeared

The problem:

This could be a result of the proteins degrading, potentially caused by issues with sample preparation and handling.

What to do:

- **Keep samples on ice:** Always work on ice throughout the lysis and sample preparation process to slow down protease activity.
- **Use fresh inhibitors:** Immediately add fresh protease and phosphatase inhibitors to your lysis buffer.
- **Minimize handling time:** Process samples quickly and keep them in a cold environment to further reduce degradation risk.

My bands are 'smiling' or distorted

The problem:

Curved or distorted bands ('smiling bands') may indicate uneven migration during electrophoresis.

What to do:

- **Adjust the voltage:** Smiling bands can indicate the voltage is too high, reduce the voltage and make sure you keep the gel cool during electrophoresis by keeping it on ice or in a cold room.
- **Check the gel polymerization:** Incomplete or uneven polymerization can lead to variations in gel density, causing bands to curve. Check your gel recipe to ensure the quantities are correct, and make sure the gel is covered entirely in buffer while setting.

My target bands are faint or missing

The problem:

If proteins do not transfer fully from the gel to the membrane, your target bands might be faint or missing entirely.

What to do:

- **Confirm the transfer success:** After transfer and before continuing the western blot protocol, check whether the proteins were transferred successfully with Ponceau S or Coomassie staining.
- **Increase the transfer time or voltage:** Adjust the transfer parameters by either extending the transfer duration or increasing the voltage (but be sure to keep everything cool).
- **Adjust the buffer composition:** Make sure the transfer buffer is freshly prepared and that its composition is optimal.
- **Check assembly:** Ensure that the transfer sandwich is assembled correctly without any air bubbles between the gel and membrane, and make sure that the transfer was performed in the right direction.

My bands are obscured and difficult to distinguish

The problem:

Excessive background noise, which can be caused by issues with blocking buffer, antibody concentrations, or washing, can make bands hard to see

What to do:

- **Optimize blocking buffer:** Experiment with different blocking agents to see which one best reduces non-specific binding.
- **Increase wash times:** Extend the washing steps to ensure that unbound antibodies are thoroughly removed.
- **Review antibody dilutions:** Ensure that the primary and secondary antibody concentrations are appropriate; too high a concentration can lead to non-specific binding.

All my bands have weak or no signal

The problem:

A weak or absent signal may be due to several factors, such as insufficient antibody binding or problems with the transfer process.

What to do:

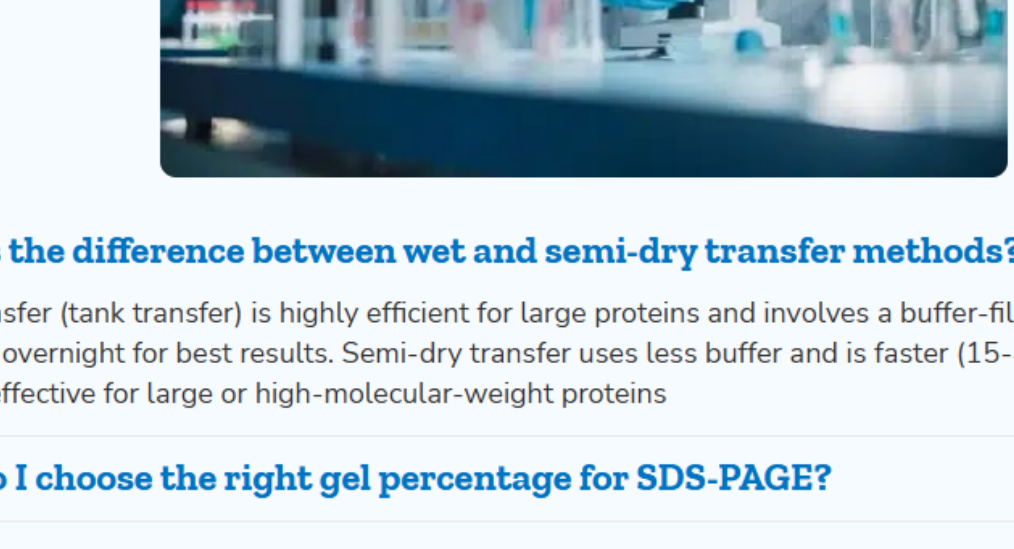
- **Adjust antibody concentrations:** Increase the concentration of the primary antibody, and increase the incubation time.
- **Confirm proper transfer:** Verify protein transfer using a reversible stain like Ponceau S.
- **Check the amount of protein:** It may be that not enough protein present so ensure that enough is loaded into each well.

Why Boston BioProducts?

Consistent buffer composition, ultrapure chemicals, and carefully formulated blocking agents can improve protein separation, enhance target detection, and reduce non-specific binding. Whether it's selecting the right lysis buffer for efficient protein extraction, using high-purity SDS sample buffers for proper denaturation, or employing optimized detection reagents for clear, high-sensitivity results, each component plays a crucial role in data quality.

By integrating Boston BioProducts' high-quality reagents into your SDS-PAGE and Western blotting protocol, you can achieve clearer, more consistent results, increasing the reliability and reproducibility of your protein analysis, and reducing troubleshooting time.

FAQs



− **What's the difference between wet and semi-dry transfer methods?**

Wet transfer (tank transfer) is highly efficient for large proteins and involves a buffer-filled chamber, taking 1-2 hours or overnight for best results. Semi-dry transfer uses less buffer and is faster (15-45 minutes), but it may be less effective for large or high-molecular-weight proteins

+ **How do I choose the right gel percentage for SDS-PAGE?**

+ **What is the best blocking buffer to use in the western blot protocol?**

+ **How do I lyse adherent and suspension cells for SDS-PAGE and western blot?**

+ **Which assay should I use to determine protein concentration?**

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