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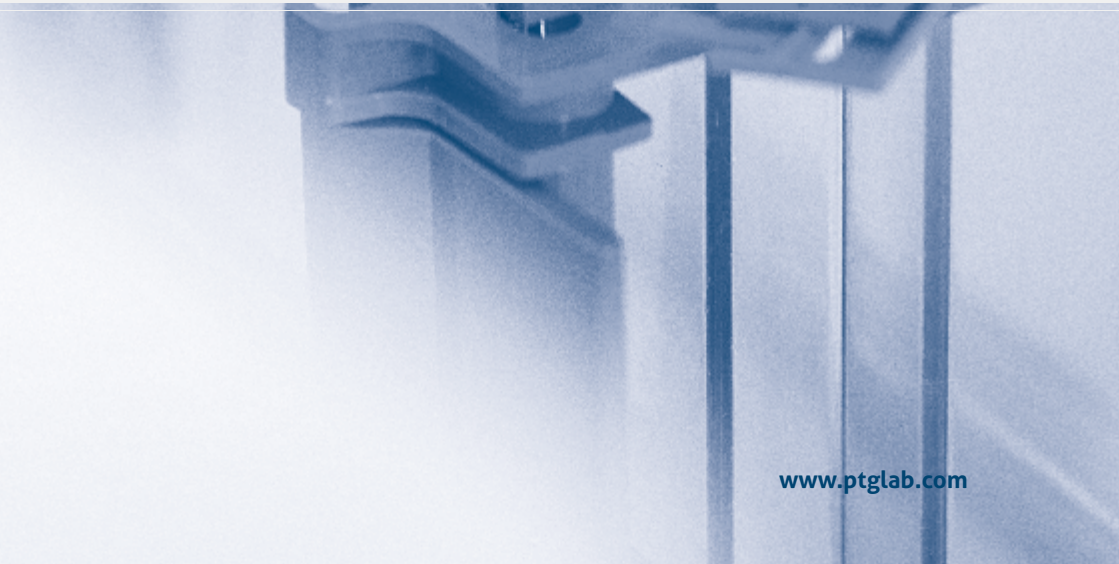
The Complete Guide To

WESTERN BLOTTING



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WELCOME

About this guide

This booklet aims to give you a complete guide on how to perform a Western blot that produces optimal results. The booklet includes original Western blot lab protocols, useful technical tips, and troubleshooting.

This guide will take you through the experiment step by step, enabling you to get the most from your research.

What's Inside

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Standard Western Blot PROTOCOL

SDS-PAGE

1. Construct an SDS-PAGE gel according to the molecular weight (MW) of your target protein(s).
   See page 12 for SDS-PAGE gel recipes.
2. Prepare samples in microfuge tubes. Add 4x SDS sample buffer so the total protein amount is 30–50µg per sample (according to the protein amount measured by Bradford or BCA protein assay).
3. Flick microfuge tubes to mix samples, spin them shortly, and then heat to 95–100°C for 5 minutes.
4. Set up electrophoresis apparatus and immerse in 1x running buffer. Remove gel combs and cleanse wells of any residual stacking gel by pipetting running buffer up and down in each well using gel-loading tip **(*Figure 1*)**.
5. Load samples and appropriate protein markers onto the gel using a tip.
6. Place the lid on the gel tank. Turn on electrophoresis power pack and set to a low voltage (as the sample runs through the stacking gel), increasing to a higher voltage (e.g., 120V) when the dye front reaches the separating layer. Stop the gel running when the dye front migrates to the desired position.

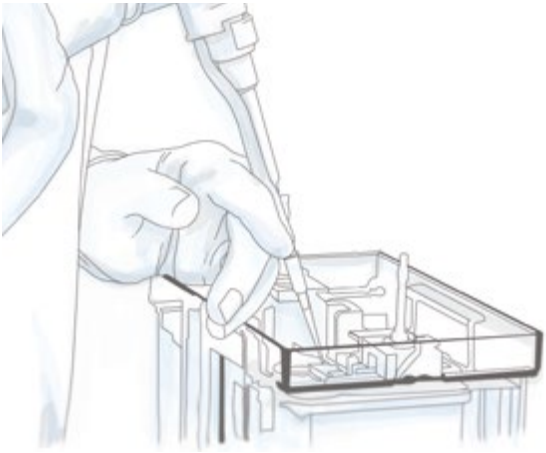
Tip: Tris-tricine gels separate low MW proteins (<20 kDa) better than Tris-glycine gels. See page 16.

Membrane Transfer

Please note: PVDF membranes (or PSQ membranes with 0.22µm micropores for targets less than <30 kDa) are strongly recommended.

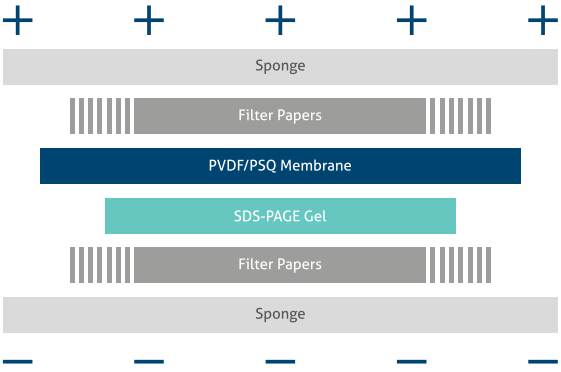
1. Soak membranes in methanol for 30 seconds before moving to transfer buffer.
2. Soak the filter papers and sponges in transfer buffer.
3. Sequentially assemble the transfer constituents according to the illustration shown on the next page *(Figure 2)*, and ensure no bubbles lie between any of the layers. Apply semi-dry or wet transfer systems according to the instructions of the blotting apparatus manufacturer.

Figure 1



After removing gel combs rinse each well thoroughly with running buffer to ensure no remaining bits of acrylamide are blocking them. Use gel-loading pipette tips to facilitate easy and even loading of your protein samples.

Figure 2



A representation of the components of a transfer "sandwich." Note the orientation of the gel and membrane: the PVDF membrane is situated nearest to the positive electrode. The binding of SDS to proteins results in the complex having an overall negative charge. Therefore, the SDS-bound proteins travel towards the positive electrode.

Immunoblotting

1. Following transfer, wash the membrane twice with distilled water.
2. Gently mark the bands of the MW ladder on the membrane using a pencil.
3. If desired, stain the membrane with commercial Ponceau red solution for 30 seconds to visualize protein bands, then wash away Ponceau red solution with generous amounts of 1x TBST.
4. Block with 1x TBST containing (2–5%) non-fat dry milk (or 1–5% BSA for the detection of phospho-epitopes) with constant rocking for 1 hour or overnight at 4°C.
5. Dilute primary antibody in blocking solution with a starting dilution ratio of 1:1000.
   (Optimal dilutions should be determined experimentally with a dilution series.) Incubate the membrane with primary antibody for 1 hour (or overnight at 4°C) on a benchtop rocker.
6. Wash membrane three times with 1x TBST for 10 minutes each.
7. Incubate the membrane with a suitable HRP-conjugated secondary antibody (recognizing the host species of the primary antibody), diluted according to the instructions. Incubate for 1 hour with constant rocking.
8. Wash membrane three times with 1x TBST for 10 minutes each.

Tip: Do not let the membrane dry at any stage of the blotting process.

Signal Detection

1. Prepare ECL substrate according to the manufacturer's instructions.
2. Incubate the membrane completely with substrate for 1–5 minutes (adjust time for more sensitive ECL substrates, e.g., SuperSignal West Femto Chemiluminescent Substrate [Pierce]).
3. Expose the membrane to autoradiography film in a darkroom or read using a chemiluminescence imaging system.
4. Line up the developed film in the correct orientation to the blot and mark the bands of the MW ladder directly onto the film. It is also advisable to add notes such as lane content, film exposure time, and ECL properties.

Tip: Use multiple exposure lengths to determine the optimal exposure time. Use fluorescent markers and clip the top-right corner of your film as a guide for blot film orientation.

Standard Western Blot PROTOCOL

Buffers Required

1x TBST (1000ml)	
20mM Tris-base	2.4 g
150mM NaCl	8.76 g
50mM KCl	3.73 g
0.2% Tween-20	2ml
Adjust pH to 7.6.	
Add ddH_2O to 1000ml	

4x SDS Sample Buffer (100ml)	
150mM Tris HCl (pH 7.0)	15ml (of a 1M stock)
25% Glycerol	25ml
12% SDS	12 g
0.05% Bromophenol Blue	0.05 g
6% ß-mercaptoethanol	6ml
Add ddH_2O to 100ml, aliquot, and store at -20°C.	

Wet Transfer Buffer (1000ml)	
25mM Tris-base	3.03 g
192mM Glycine	14.4 g
20% Methanol	200ml
Add ddH_2O to 1000ml.	

Semi-dry Transfer Buffer (1000ml)	
48mM Tris-base	5.81 g
39mM Glycine	2.93 g
0.0375% SDS	0.375 g
20% Methanol	200ml
Add ddH_2O to 1000ml	

How To Optimize Your WESTERN BLOT

Obtaining perfect, publication-ready results straightaway is not exactly the norm when performing Western blotting. However, there are steps you can take to refine your blotting experiments and ensure they get off to the best start possible.

1. SDS-PAGE

Your Western blotting experiments start long before you begin to work with your membrane. If your gel is sloppily made or you run it at an excessive voltage, your final results will suffer. If you cast your own gels, take extra care to ensure uniformity in their makeup. Select the right acrylamide percentage and avoid that dye-front "smiling" effect by resisting the temptation to run your gels at high voltages.

Above all, load a uniform amount of protein; the Proteintech® validation lab finds that 0–50ug of protein is a suitable amount for the detection of most proteins, and usually gives streak-free and well-separated bands.

2. Membrane

Membrane choice can make a huge difference to the outcome of your Western blotting experiments. The two main membrane types are PVDF and nitrocellulose, but there are now several versions of each on the consumables market.

PVDF tends to be good for lowly expressed proteins, but high background and it works better with hydrophilic/polar/charged target antigens. Nitrocellulose membranes are good for normal or highly expressed proteins and it works better with hydrophobic/non-polar antigens.

3. Antibody Concentration

Your next step should be to determine the optimum working antibody concentration. The rate of binding between antibody and antigen is affected by their relative concentrations in solution (among other variables). Often you will need to tweak the antibody concentration to get better blots. This step is called antibody titration, and it should be performed every time a new antibody or new set of experimental conditions is used.

"Often you will need to tweak the antibody concentration to get better blots."

"Remember that milk-based blocking buffers contain such things as endogenous biotin, glycoproteins, and enzymes that may interfere with the signal."

4. Blocking Conditions

Various blocking buffers are available and not all of them work in every situation. It is important to try several blocking buffers for each antibody-antigen pair. Remember that milk-based blocking buffers contain such things as endogenous biotin, glycoproteins, and enzymes that may interfere with the signal. Milk-based buffers, for example, contain active phosphatases that will sabotage your detection of phosphorylated proteins; in these cases, try alternatives such as bovine serum albumin-based blockers.

5. Washing

If the Western blot shows high background signal, revising the washing steps on the next run-through has proven to be useful. Increasing washing times and volumes will reduce background signal along with performing additional buffer changes or using a stronger detergent (e.g., SDS instead of Tween 20).

6. Detection

The detection stage is integral to obtaining a good Western blot signal. There are many chemiluminescent reagents and chemiluminescent alternatives out there, such as fluorescent detection—so there are plenty of options if your current detection method isn't meeting lab standards. You can choose reagents with more sensitivity or those that produce longer-lasting signal, for example. The latter choice will increase the reproducibility of blots immeasurably.

A simpler method is to experiment with film-exposure times. This is probably the optimization step performed most frequently, as it is quick and easy. Always expose your blots for a range of individual time points.

SDS-PAGE GEL RECIPES

In order to target proteins with MWs between 20 and 200 kDa, you will need to create a conventional SDS-PAGE gel using the recipes shown below. The percentage of gel you require corresponds with the MW of your target protein.

Recipe 1

Separating Gel (mls, total 10ml)				
MW of target protein (kDa)	80-200	35-100	25-60	20-40
Gel Percentage	8%	10%	12%	15%
ddH_2O	2.1	1.5	0.8	0
30% Acrylamide	2.7	3.3	4	5
2x Separating Buffer	5.0	5.0	5.0	5.0
10% APS	0.1	0.1	0.1	0.1
TEMED	0.01	0.01	0.01	0.01

Stacking Gel (mls)			
	4ml	6ml	8ml
MW of target protein (kDa)	—	—	—
Gel Percentage	4%	4%	4%
ddH_2O	1.4	2.1	2.7
30% Acrylamide	0.5	0.8	1.1
2x Stacking Buffer	2.0	3.0	4.0
10% APS	0.04	0.06	0.08
TEMED	0.004	0.006	0.008

Recipe 2

2x Separating Buffer Recipe (makes 1000ml)	
Tris HCl (pH 8.8)	90.8 g
SDS	2.0 g
Dissolve compounds thoroughly. Adjust pH slowly to pH 8.8 with concentrated HCl, then add ddH_2O to 1000ml.	

Recipe 3

2x Stacking Buffer Recipe (makes 1000ml)	
Tris HCl (pH 6.8)	30.35 g
SDS	2.0 g
Dissolve compounds thoroughly. Adjust pH slowly to pH 6.8 with concentrated HCl, then add ddH_2O to 1000ml.	

Recipe 4

1x Running Buffer Recipe (makes 1000ml)	
Tris-base	1.51 g
Glycine	7.5 g
SDS	0.5 g
Dissolve compounds thoroughly, then add ddH_2O to 1000ml.	

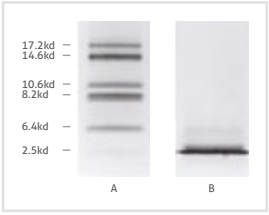
How To Optimize Your Results With LOW MW PROTEINS

At extreme ends of the Molecular weight (MW) spectrum, regular SDS-PAGE and Western blotting techniques suffer from limitations: poor separation, signal reduction, or even a total absence of target bands. Low MW proteins (<20 kDa) are challenging to detect, however several protocol modifications can be employed when handling these proteins to improve their retention and resolution.

Use Tricine

In general, glycine gels are ideal for resolving any proteins that fall within the MW range of 30–250 kDa. However, Acrylamide gels based on a Tris-Tricine buffer system will greatly improve your chances of "seeing" your target band(s) if you are working below the 30 kDa range.

You can find a recipe for a 15% Tricine gel on page 16.



Comparison of Tricine- (A) and glycine-SDS-PAGE (B) separation of myoglobin fragments. Adapted from Schägger and von Jagow 1987.

Why It Works

The difference in separation capabilities of glycine and tricine gels is attributed to the differing properties of the glycine and tricine compounds, such as their pK values and ionic mobility. Tricine is more optimal for the separation of low MW proteins because it "stacks" proteins into more uniform bands. A tricine-based stacking layer shifts the upper stacking limit down to as low as 30 kDa for the first stack,[1] preventing overloading at the interface between the gel layers.

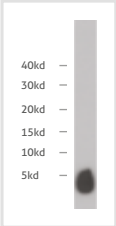
[1] *H Schägger and G von Jagow. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem. 1987;166(2):368–79.*

Protein Transfer

As well as ensuring the optimum separation of low MW proteins, you also need to take care at the protein transfer stage. Low MW proteins are susceptible to "over transfer" – loss of sample due to lack of retention by the transfer membrane and/or too-rapid transfer.

Membrane Choice and Pore Size

Most labs have a preferred choice of Western blotting membrane, however PVDF is a better choice in the case of smaller, low MW proteins, due to its greater capacity to bind proteins. There are various membrane pore sizes on offer – opt for a smaller pore size to obtain better transfers of your low MW target proteins.



The Proteintech® lab detected 4 kDa sarcolipin protein with anti-sarcoplin antibody (18395-1-AP; 1:300), following separation on a 15% Tricine gel and protein transfer to 0.22 µm pore size PVDF membrane.

Transfer Conditions

In addition to membrane choice, factors such as transfer system, length, temperature, and buffer composition come into play when dealing with low MW proteins. In the case of small proteins, less is often more when it comes to transfer length and voltage. How much you should adjust these by will be down to the brand and model of system being used.

Other Tips

Be aware some proteins will escape the dye front of the sample loading buffer.

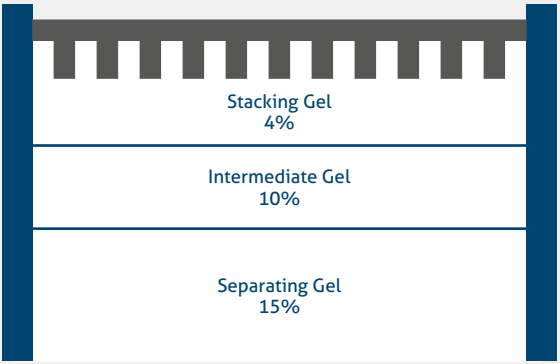
You can choose to soak your gel in an SDS-free buffer (or simply H_2O) for 5 minutes prior to setting up your transfer. This helps to remove SDS, which coats small proteins and protein fragments in negative charges, increasing their rate of passage through the Western blot membrane.

Tricine Gel Recipe For LOW MW PROTEINS

For target proteins with MWs of less than 20 kDa, a tricine gel system will obtain higher resolution and is highly recommended. Make three layers of tricine gels as laid out in the following table and diagram. Apply specific tricine gel running buffer to the running system and perform transfer as usual.

Recipe

Items	Separation	Intermediate	Stacking
Gel Percentage	15%	10%	4%
Gel Volume	6 ml	3 ml	2 ml
38% Glycerol	1.6	—	—
ddH_2O	—	1.2	1.4
30% Acrylamide	2.7	0.8	0.3
3.0 M Tris HCl (pH 8.5)	2.14	1	—
1.0 M Tris HCl (pH 6.8)	—	—	0.3
10% SDS	0.06	0.03	0.02
10% APS	0.06	0.03	0.02
TEMED	0.003	0.003	0.002



Choosing The Right LYSIS BUFFER

Preparing Protein Lysates

Cell lysis is the breaking down of the cell membrane and the separation of proteins from the non-soluble parts of the cell. Lysate buffers contain different detergents that help to release soluble proteins (Triton-X, Tween, SDS, CHAPS). Dependent on the location of the protein of interest, a different lysate buffer is needed to obtain a high yield and purity of the protein.

However, every protein is different and may react differently with the buffers and detergents. If you don't get your protein of interest in solution or you are studying a special protein–protein interaction, you can try different buffers and exchange the detergents.

General Guidelines

Whole-cell lysate/membrane-bound proteins

The most commonly used buffers are RIPA and NP-40. RIPA buffer's harsh properties are best suited for hard-to-solubilize proteins.

RIPA: 25mM Tris, HCl (pH 7.6) 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS

NP-40: 50 mM Tris, HCl (pH 8.5) 150 mM NaCl, 1% detergent

Nuclear/mitochondria proteins

RIPA is the preferred choice here. However, fractions protocols are often used to increase the concentration of the desired protein.

Cytoplasmic proteins

A Tris-HCl lysis sometimes shows advantages over RIPA buffer. Optimal conditions should be tested for the protein of interest.

Don't forget your inhibitors

Denaturation/proteolysis and dephosphorylationen (in case of phospoproteins) should always be kept to a minimum and added freshly to the cell lysate (EDTA, sodium orthovanadate, PSMF, Aprotinin).

TROUBLESHOOTING

Overcome your Western blot difficulties with our troubleshooting advice, covering problems such as weak/no signal, non-specific bands, high signal, and other common issues.

Weak/No Signal

Issues with the primary and/or secondary antibody

- Titrate the antibody to determine optimum concentration.
- The antibody may have lost activity – perform a dot blot to determine activity and optimal concentration.
- Include a positive control (e.g., overexpressed protein, purified protein, positive cell line, etc. Adjust protein loading accordingly).
- Change incubation time and temperature (4°C, overnight).

Target protein abundance is too low

- Load more protein per well.
- Enrich low-abundance proteins by immunoprecipitation, fractionation, etc.
- Use appropriate treatment to induce target protein expression or modification.
- Ensure sample has not degraded.
- Include protease inhibitors in the lysis buffer.
- Use the optimum lysis buffer for the target protein's subcellular localization.
- Check protein loading with an internal loading control antibody.

Membrane choice

- Select PVDF or NC membranes based on hydrophobicity/hydrophilicity of the target antigen.
  - Check the hydrophobicity/hydrophilicity of the antigen sequence.
  - PVDF membrane works better with hydrophilic/polar/charged target antigens.
  - Nitrocellulose works better with hydrophobic/non-polar antigens.

Blocking buffer issues

- Blocking for too long can mask specific epitopes and prevent antibody binding.
- Reduce the percentage of, or remove, the blocking reagent from the antibody incubation buffers.
- Switch to using an alternative blocking reagent.

Low molecular weight targets

- Use a Tris-tricine gel for protein targets <20 kDa.

  (See page 16 for Tricine gel recipe).
- Reduce transfer times and/or use smaller pore size membranes (0.22 µm) for low MW proteins <30 kDa.

  (See page 14 for How To Optimize Your Results With Low MW Proteins).
- Wet transfer is recommended for small proteins (<10 kDa).

Unsuccessful transfer

- Ensure proper transfer set-up (e.g., no air bubbles trapped between the gel and the membrane).
- Thicker gels can result in incomplete transfer of high-molecular-weight-proteins.
- Check the quality of protein transfer with a reversible, universal protein stain, e.g., Ponceau-S.
- Wet transfer produces higher-resolution transfers over semi-dry transfer.

Sodium azide contamination

- The presence of sodium azide inhibits the activity of HRP.
- Use sodium azide-free buffers.
- Ensure sufficient washing.

Film exposure too short/ Detection reagent not sensitive enough

- Check several exposure times to achieve optimum detection.
- Try different detection reagent compositions and/or brands.
- Dilute chemiluminescent reagents in high-purity water.

High Background
(Uniform Distribution)

Antibody concentration too high
- Use higher antibody dilution.

Insufficient blocking
- Increase the concentration of blocking reagent (e.g., from 5 to 7%).
- Increase blocking time and/or temperature.
- Add Tween 20 to the blocking buffer.
- Include blocking reagent and Tween 20 in the primary antibody dilution buffer.

Inadequate washing
- Increase washing time and volume.

Dry membrane
- Ensure membrane does not dry out during the immunoblotting process.

Non-specific binding of secondary antibody
- Perform a secondary antibody-only control experiment (omit the primary incubation step).
- Use a pre-adsorbed secondary antibody with reduced cross-reactivity to unwanted species.
- For phosphorylated protein detection, do not use milk-based buffers such as non-fat milk or casein buffer. (Milk and casein are phosphoprotein-rich.)

Film exposure too long/ Detection reagent too sensitive
- Check different types and dilution of the detection reagent.

High Background
(Non-Specific Bands)

The troubleshooting tips for high background (uniform distribution) can also be applied to scenarios where non-specific but distinct bands appear on the Western blot membrane.

The following should also be considered:

Target protein abundance is lower than threshold of non-specific binding

- Load more protein per well at SDS-PAGE.
- Enrich low-abundance proteins by immunoprecipitation, fractionation, etc.

Sample degradation

- Prepare fresh lysates each time
- Use freshly prepared sample kept on ice up until the addition of sample buffer and immediate heating to 95°C for 5–10 minutes.
- Tissue extracts tend to produce more non-specific bands and degradation products. Use fresh, sonicated, and clarified tissue extracts.
- Always include protease inhibitors (and phosphatase inhibitors for the detection of phosphorylated targets).
- Ensure sample has not degraded.

Interference from other isoforms

- Check for the presence of known isoforms in the literature or at uniprot.org.
- Use an isoform-specific antibody.

Inefficient SDS-PAGE separation

- Change the gel percentage to suit the target protein's MW.
- Lower percentage Tris-Glycine gels should be used for larger proteins, or use Tris-Acetate-based gels and buffers.

  (See page 12 for our SDS-PAGE gel recipes).
- Higher percentage Tris-Glycine gels (up to 15%) should be used for smaller proteins (<20 kDa) or use Tris-Tricine gels.

  (See page 16 for our Tricine gel recipe).

Presence of post-translational modifications

- Know your protein of interest, band sizes can shift due to glycosylation, phosphorylation, precursor maturation, etc.

Lack of controls

While the omission of control samples from a Western blot is not a cause of non-specific bands, their inclusion can tell you why you may be seeing them on your membrane.

Controls you could include are:

- **Positive controls:**
  - Samples from cells overexpressing the target protein.
  - Purified recombinant protein.
  - Cell line/tissue with proven positive signal.
- **Negative controls:**
  - Samples targeted with RNA interference.
  - Samples from knockout tissues/cells.
  - Cell line/tissue with proven negative signal.

Other Blotting Issues

Ghost hollow bands

- This happens when the ECL substrate is used up too rapidly.

Sample overloading

- Decrease the total protein loading for each sample.

Too much antibody

- Decrease the concentrations of the primary and/or secondary antibodies.

Inverse staining (i.e., white bands on a dark blot)

- Too much primary and/or too much secondary antibody.
- Use antibodies at higher dilutions.

Molecular weight marker staining

- The antibody reacts with the MW marker.
- Add a blank lane between the MW marker and the first sample lane.

"Smiling" bands

- Migration through the gel was too hot or too fast.
- Reduce the voltage applied to run the SDS-PAGE gel or run the gel in a cold room.

Uneven bands

- High protein concentrations can result in diffuse protein bands.
- Uneven protein loading: assay protein samples and load by protein amount. Check for even protein loading by stripping and reprobing the blot with an internal control antibody (or use an HRP-conjugated loading control antibody).
- Uneven gel composition (gel has set too quickly while casting or buffer was mixed inadequately).
- Uneven bands can be due to insufficient buffer being added to the tank during running.

Blank areas/white spots

- Can be caused by improper/uneven transfer or air bubbles.

Dark spots/dots

- This problem can be caused by antibodies binding to the blocking reagent in the blocking buffer.
- Change to another blocking reagent.
- Filter the blocking buffer.
- Wash excess detection reagent from the membrane before exposure.

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PROMOTION

TAKE CONTROL OF YOUR WESTERN BLOTTING

Proteintech control antibodies just **$99 each**!

The proteins that normally serve as internal controls in your research may not always take center stage, but Proteintech understand they are indispensable for conducting meaningful research, most notably for the proper interpretation of Western blots.

For more details on this promotional offer and a full list of included antibodies, visit the Proteintech website.

www.ptglab.com

Antibodies Included:

Antibody Name		Catalog Number	Application
alpha Tubulin	33	66031-1-Ig	ELISA, FC, IF, IHC, IP, WB
beta Actin	553	60008-1-Ig	ELISA, FC, IF, IHC, WB
GAPDH	424	60004-1-Ig	ELISA, FC, IF, IHC, IP, WB
6*His, His Tag	64	66005-1-Ig	ELISA, IF, IP, WB
GST-TAG	12	66001-1-Ig	ELISA, IP, WB

The above is a small selection of the antibodies available at the discounted price. A full list of over 50 antibodies included can be found at ptglab.com.

00 This number shows the amount of times our antibody has been cited in a publication.

Loading Control ANTIBODIES

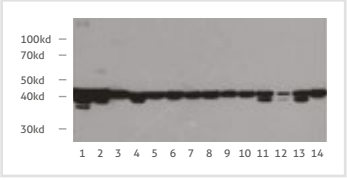
Western blotting requires loading controls, they are used for the semi-quantification of protein levels to ensure that the observed alterations in target proteins are actually due to experimental manipulations. Internal control proteins, i.e., those with constant, unchanged levels, are usually detected in a second round of blotting, following primary detection of your protein of interest. This step is used to standardize results and normalize for any errors that creep into a Western blot experiment. The loading control candidates for Western blotting are usually proteins with high, constitutive, and unchanged expression throughout an experiment, regardless of tissues or cell types. Control candidates require careful selection as they can be affected by the conditions of your experiment. Here, we've provided background information on a selection of the internal control proteins targeted by the control antibodies we offer.

Actin

Recommended For:
Whole cell/cytoplasma

Molecular Weight
~42 kDa

The six isoforms of actin constitute a family of highly conserved globular proteins comprising three main isoform groups: alpha, beta, and gamma. The alpha actins are a major constituent of the contractile apparatus in muscle tissues. The beta and gamma actins coexist in most cell types and are an integral part of the cytoskeleton. Together, actins are the most abundant proteins in the typical eukaryotic cell, accounting for about 15 percent of total protein in some cell types. As such, actin is widely used as an internal control in Western blotting experiments.

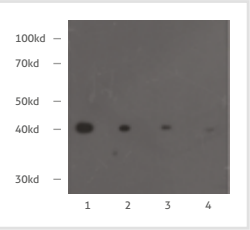


Key

Lane 1: Zebra Fish
Lane 2: Human Placenta
Lane 3: Human Heart
Lane 4: Human Brain
Lane 5: SW1900 Cell Line
Lane 6: A2780 Cell Line
Lane 7: PC-3 Cell Line
Lane 8: HepG2 Cell Line
Lane 9: HEK293 Cell Line
Lane 10: HeLa Cell Line
Lane 11: NIH3T3 Cell Line
Lane 12: SP2/0 Cell Line
Lane 13: Raw264.7 Cell Line
Lane 14: C6 Cell Line

Western blot analysis of ACTB in multiple cell lines and tissue lysates using anti-beta actin mouse monoclonal (60008-1-Ig) antibody at a dilution of 1:5000.

Proteintech®'s polyclonal ß-actin antibody (20536-1-AP) was generated using a ß-actin protein antigen (amino acids 14–167). It recognizes all forms of actin, making it a pan-actin antibody. However, if your studies involve work with skeletal muscle samples, or you are working with conditions that see changes in cell growth or altered interactions with the extracellular matrix, another loading control may be better suited to your needs.



Key
Lane 1: 1:500
Lane 2: 1:1000
Lane 3: 1:2000
Lane 4: 1:4000

HeLa cell lysate (10 ug/lane) was separated by SDS-PAGE and actin was detected by anti-ß-actin antibody 20536-1-AP at varying dilutions. (L–R) 1:500, 1:1,000, 1:2,000, and 1:4,000.

Actin Antibodies

Antibody Name	Cat No	Type
ß-actin	23 20536-1-AP	Rabbit Poly
ß-actin	220 60008-1-Ig	Mouse Mono

Related Antibodies

Antibody Name	Cat No	Type
ACTA1	17521-1-AP	Rabbit Poly
α-SMA	4 55135-1-AP	Rabbit Poly
HRP-conjugated ß-actin	HRP-60008	Mouse Mono

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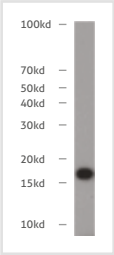
COX-4

Recommended For:
Mitochondrial

Molecular Weight
17 kDa

COX-4, or COXIV (cytochrome c oxidase subunit IV), is a nuclear-encoded subunit of the human mitochondrial respiratory chain enzyme cytochrome c oxidase (COX). The COX-4 subunit can be expressed as either of two isoforms. Because of its dependably high level, COX4I1 is commonly detected as an effective loading control for mitochondrial proteins. However, some caution is advised when selecting this protein for Western blot detection as many other proteins run at its 17 kDa size during SDS-PAGE (make sure your band of interest won't be obscured). For an alternative mitochondrial protein loading control, see our entry on VDAC1 below.



MCF7 cells were subjected to SDS PAGE followed by Western blot with 11242-1-AP(COXIV antibody) at a dilution of 1:1000.

COX-4 Antibodies

Antibody Name	Cat No	Type
COX4I1	12 11242-1-AP	Rabbit Poly
COX4I1	66110-1-Ig	Mouse Mono
COX4I2	5 11463-1-AP	Rabbit Poly

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"However, some caution is advised when selecting this protein for Western blot detection as many other proteins run at its 17 kDa size during SDS-PAGE."

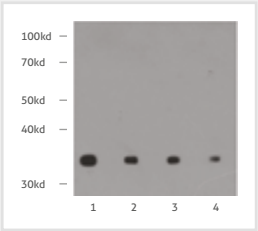
GAPDH

Recommended For:
Whole cell/cytoplasma

Molecular Weight
36 kDa

Glyceraldehyde 3-phosphate dehydrogenase, commonly known as GAPDH, catalyzes the six steps of glycolysis. It also participates in nuclear events. Its expression is high and constant in most tissues and cell types. GAPDH is commonly used as a protein loading control in Western blotting and internal control for RT-PCR. However, the amount of GAPDH expression can differ between some tissues. It is also worth noting that some physiological factors, such as hypoxia and diabetes, increase GAPDH expression in certain cell and tissue types.

Proteintech® has both monoclonal GAPDH (60004-1-Ig) and polyclonal GAPDH (10494-1-AP) antibodies available, both raised against a whole-protein antigen (amino acids 1–335) of human origin.



Western blot with HeLa cell lysate using anti-GAPDH (10494-1-AP) at various dilutions. (L–R) 1:2000, 1:4000, 1:8000, and 1:16000.

GAPDH Antibodies

Antibody Name	Cat No	Type
GAPDH	10494-1-AP	Rabbit Poly
GAPDH	60004-1-Ig	Mouse Mono

Related Antibodies

Antibody Name	Cat No	Type
HRP-conjugated GAPDH	HRP-60004	Mouse Mono

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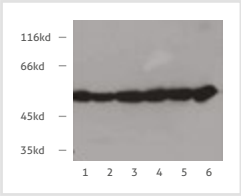
Tubulin

Recommended For:
Whole cell/cytoplasma

Molecular Weight
50–55 kDa

Tubulins are the major components of microtubules. The Microtubules are involved in a wide variety of cellular activities. They are highly and stably expressed and conserved across the species, and tubulins make excellent whole-cell or cytoplasmic fraction loading controls. However, tubulin expression may vary according to resistance to antimicrobial and antimitotic drugs.

Proteintech® has polyclonal antibodies against several tubulin subunits, including an α-tubulin antibody (11224-1-AP) and two ß-tubulin antibodies, 10068-1-AP and 10094-1-AP.



Key
Lane 1: HeLa Cell
Lane 2: NIH3T3 Cell
Lane 3: 293 Cell
Lane 4: A431 Cell
Lane 5: Jurkat Cell
Lane 6: Raji Cell

Western blot on multiple cells with anti-tubulin-alpha (11224-1-AP) at a dilution of 1:1,000.

Tubulin Antibodies

Antibody Name	Cat No	Type
Alpha Tubulin	21 11224-1-AP	Rabbit Poly
Beta Tubulin	5 10068-1-AP	Rabbit Poly
Beta Tubulin	13 10094-1-AP	Rabbit Poly

Related Antibodies

Antibody Name	Cat No	Type
Alpha Tubulin	11 66031-1-Ig	Mouse Mono
HRP-conjugated TUB1A	HRP-66031	Mouse Mono

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