Distorted Pattern

Western Blot Procedural Step

01 SDS-PAGE Electrophoresis

Problem

a. Uneven band sizes
b. Smiling effect on bands
c. Diffuse bands
d. Inadequate separation

Possible Solution(s)

a
- Ensure that the APS and TEMED used for gel polymerization are properly added and mixed (and not expired).
- Ensure that the gel has not dried out during storage.

b
- Gel temperature during electrophoresis is too high and unevenly distributed over gel. Try decreasing voltage or cooling the gel during the run.
- Load empty lanes with SDS-PAGE sample buffer.

c
- Reduce the amount of protein loaded.
- Ensure that the proteins are completely reduced and denatured, unless native protein gel is being used.

d
- Increase or decrease the gel percentage for small proteins or large proteins, respectively.
- Different gels and associated buffers are recommended as follows:
  - For general use:
    Tris-Glycine gel with Tris-Glycine running buffer.
  - For small-to-medium protein size:
    Bis-Tris gel with MES SDS running buffer.
  - For medium-to-large protein size:
    Bis-Tris gel with MOPS SDS running buffer.
  - For large protein size:
    Tris-Acetate gel with Tris-Acetate running buffer.

Streaky Bands

Western Blot Procedural Step

	Problem	Possible Solution(s)
01 Sample Preparation	a. Impurities in the sample b. Improper sample preparation	01 a • Re-spin the sample. 01 b • Perform total protein or subcellular fraction preparation rapidly with an optimized extraction kit (e.g., Trident Membrane Protein Extraction Kit (GTX16373)).
02 SDS-PAGE Electrophoresis	Protein overloaded	02 • Accurately measure protein concentration and load an appropriate amount sufficient to observe a specific signal.

Black Dots

Western Blot Procedural Step

01 Blocking

Problem

Particles of blocking reagents are bound to antibodies

Possible Solution(s)

01
- Ensure that the blocking reagents are completely dissolved, or filter the blocking agent if necessary.
- Change blocking buffer.

White Bands

Western Blot Procedural Step

	Problem	Possible Solution(s)
01 SDS-PAGE Electrophoresis	Proteins are overloaded	01 • Reduce the amount of protein loaded.
02 Primary Antibody Secondary Antibody	Too much antibody used	02 • Reduce the amount of antibody.
03 ECL	Signal is overdeveloped	03 • Shorten exposure time. • Switch to picogram-grade ECL detection kit.

WB analysis using Trident ECL plus (GTX400006) with various sample amounts and exposure times as indicated below.

Exposure time: 18 seconds, 90 seconds, and 180 seconds.
Recombinant protein is loaded as follows:

A: 50 ng B: 25 ng C: 12.5 ng D: 6.25 ng
E: 3.13 ng F: 1.56 ng G: 780 pg H: 390 pg
I: 195 pg J: 97.5 pg

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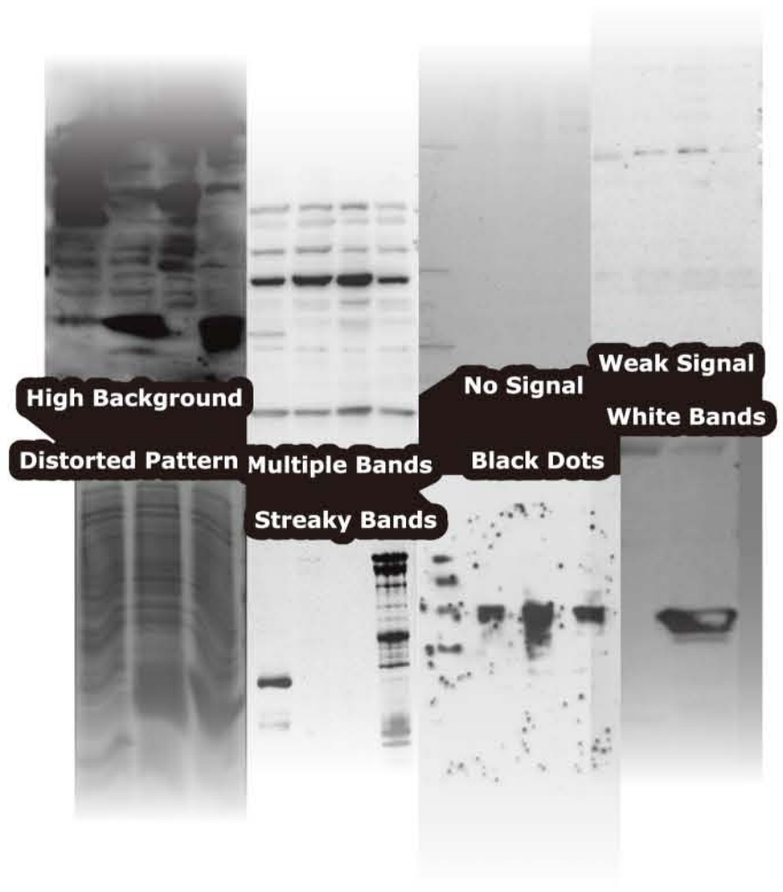
Western Blot Troubleshooting

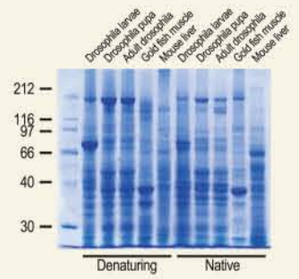


Immunoblotting, also commonly referred to as western blotting, is a fundamental technique performed in research labs all over the world. While each lab has its own protocol, the basic procedure remains largely unchanged. Nevertheless, familiarity with an application does not guarantee clear, publishable results. Obtaining pristine signals with minimal or no background often requires systematic troubleshooting that can frequently improve an initially poor result. To this end, we would like to present a list of common problems that can arise throughout the immunoblotting process and offer some possible solutions.

High Background or Multiple Bands

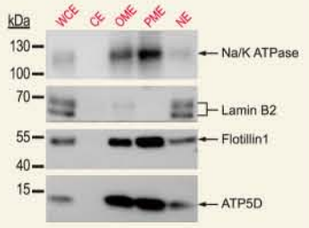
"Trident Total Protein Extraction Kit (GTX16372) is for quick extraction of total proteins from animal tissues.

- Extremely fast (1-8 minutes)
- High yield and high concentration (2-8 mg/ml)
- Ideal for small volume samples (15-20 mg of tissues)
- Ideal for either denatured or native protein extraction



"The Trident Membrane Protein Extraction Kit (GTX16373) is for rapid extraction of native total membrane proteins (organelle membrane proteins) and native plasma membrane proteins from cultured mammalian cells or tissues.

- Simple and user-friendly
- Wide range of starting cells (1 - 50 million / sample)
- Free of detergent and EDTA
- No Dounce homogenizer or tissue blender needed
- Procedure can be performed in less than 45 minutes
- High yield



Possible Solution(s)

01
- Use freshly added dithiothreitol (DTT) or 2-Mercaptoethanol (2-ME) and boil samples sufficiently to ensure protein denaturation.
- Perform native protein extraction in a detergent-free system (e.g., Trident Total Protein Extraction Kit (GTX16372)).
- Check isoforms/alternative splicing.
- Check post-translational modifications.

02
- Prepare fresh tissue extracts/lysates in appropriate buffers that contain protease inhibitors and phosphatase inhibitors.
- Keep samples on ice during the entire lysate/extract preparation procedure.
- Aliquot samples and avoid repeated freeze/thaw cycles.
- Perform total protein or subcellular fraction preparation rapidly with an optimized extraction kit (e.g., Trident Membrane Protein Extraction Kit (GTX16373)).

03
- Accurately measure protein concentration and load an appropriate amount sufficient to observe a specific signal.

04
- Change to nitrocellulose membrane (PVDF membrane is more sensitive for overall signal development than nitrocellulose membrane).

05
- Increase blocking time.
- Determine suitable blocking buffer (5% nonfat milk or 5% BSA) or use an optimized blocking buffer system (e.g., Trident Universal Protein Blocking Reagent (GTX30963)).

06
- Consult the product datasheet for the recommended dilution.
- Incubate primary antibody overnight at 4˚C in TBST buffer with blocking reagent or in an optimized blocking buffer system.
- Choose antigen affinity-purified antibodies (e.g., GeneTex antibodies).

07
- Increase wash duration.
- Wash with 0.1% Tween 20-containing buffers (e.g., Trident 10X TBST (GTX30976)).

08
- Test serial dilutions of the secondary antibody to optimize the concentration used.
- Dilute the secondary antibody in a suitable blocking buffer (5% nonfat milk or 5% BSA in TBST) or an optimized blocking buffer system.

09
- Optimize film exposure time.
- Use chemiluminescence imaging system to assess signal intensity over time.

Cat. No.	Product	Package
GTX400005	Trident RIPA Lysis Buffer	100 ml
GTX16372	Trident Total Protein Extraction Kit	5/20 Tests
GTX16373	Trident Membrane Protein Extraction Kit	5/20 Tests
GTX16374	Trident Nuclear Protein Extraction Kit	4/20 Tests
GTX30963	Trident Universal Protein Blocking Reagent (animal serum free)	100 ml
GTX30976	Trident 10X TBST	100 ml
GTX48886	Trident TBS (tablets)	100 tablets

Western Blot Procedural Step

Step	Problem	Problem
01 Protein Biology	Protein structure and modifications	a Basal level of target protein is low b Target protein is absent in the tested samples
02 Sample Preparation	Protein degradation	Protein degradation
03 SDS-PAGE Electrophoresis	Protein overloaded	Insufficient protein loaded onto SDS-PAGE gel
04 Transfer	Improper membrane used	Incomplete transfer *Confirm successful protein transfer by Ponceau S, Amido Black or India Ink staining of filter. *Assess successful protein transfer by using prestained protein markers (see GTX50875 and GTX16376).
05 Blocking	Inadequate blocking	Over-blocking
06 Primary Antibody	Non-specific binding of primary antibody	a Protein of interest may not be detected b Insufficient antibody
07 Wash	Inadequate washing	Excessive washing
08 Secondary Antibody	Non-specific binding of secondary antibody *Check by performing a blot with secondary antibody only (without primary antibody).	a Secondary antibody incompatible with the primary antibody b Insufficient antibody
09 ECL	Overexposure	Insufficient signal development

Weak or No Signal

Possible Solution(s)

01 a
- Increase the amount of protein sample loaded.
- Induce protein expression via stimulus.
- Enrich protein by using samples from specific subcellular fractions (e.g., nuclear fraction (GTX16374) versus whole cell extract (GTX16372)).
- Enrich protein amount in samples by immunoprecipitation.

01 b
- Consult antibody datasheet for a recommended positive control sample for the target protein.

02
- Prepare fresh tissue extracts/lysates in appropriate buffers that contain protease inhibitors and phosphatase inhibitors.
- Keep samples on ice during the entire lysate/extract preparation procedure.
- Aliquot samples and avoid repeated freeze/thaw cycles.
- Perform total protein or subcellular fraction preparation rapidly with an optimized extraction kit (e.g., Trident Membrane Protein Extraction Kit (GTX16373)).

03
- Accurately measure protein concentration.
- At least 20-30 µg of total protein is generally recommended.
- Ensure that correct composition of SDS-PAGE sample buffer is used.

04
- If using PVDF membrane, ensure that the membrane is methanol-activated and remains wet during incubation (always handle with clean gloves).
- If using nitrocellulose membrane, pre-soak the membrane in transfer buffer (always handle with clean gloves).
- For large target proteins, more transfer time and/or low-percentage acrylamide gels are required to increase transfer efficiency.
- For small target proteins, a 0.2 µm pore size PVDF membrane is recommended. Avoid over-transfer by reducing voltage or transfer time.

05
- Block for 1-3 hours at RT.
- Reduce the amount of blocking reagents in blocking buffer or change the blocking reagent.
- Use an optimized blocking buffer system (e.g., Trident Universal Protein Blocking Reagent (GTX30963)).

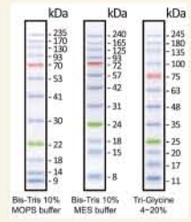
06 a
- Consult the datasheet to ensure that the protein of interest can be detected (i.e., is antibody isoform- or PTM-specific?). Perform blot with the recommended positive control.

06 b
- Avoid freeze/thaw cycles. Perform dot blot to confirm antibody's activity.
- Check if antibody is expired.
- Increase antibody concentration.
- Extend incubation time to overnight at 4˚C.
- Use signal enhancer kit (e.g., SignalPlus Antibody Enhancer (for Western Blot) (GTX49999)).

07
- Decrease wash duration.

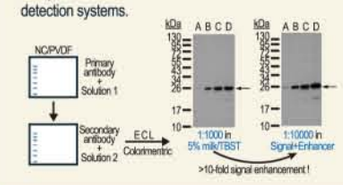
"The Trident Prestained Protein Ladder (GTX50875) is a three-color protein standard with twelve pre-stained proteins covering a wide range of molecular weights (~10 to 245 kDa).

- Ready to use.
- Wide range of molecular weights.
- 2 µl per well for general western blots.
- Two reference bands in red and green.
- Compatible with multiple buffer systems.
- Compatible with nylon, nitrocellulose, and PVDF membranes.



"Dilute your antibodies with SignalPlus Antibody Enhancer (for Western Blot) (GTX49999) instead of conventional diluents (such as TBST) and witness a remarkable increase in the ability to detect the protein of interest and to eliminate undesired background.

- Ready to use.
- >10-fold signal enhancement.
- Improve your antibody specificity.
- Compatible with nitrocellulose and PVDF membranes.
- Compatible with both chemiluminescence and colorimetric detection systems.



08 a
- Be sure to use a secondary antibody against the species in which the primary antibody was raised.
- Use a secondary antibody against the correct isotype of the primary antibody (commonly used isotypes are IgG1, IgG2, and IgM).

08 b
- See solutions suggested for primary antibody (above).

09
- Use femtogram-grade ECL detection system.
- Confirm substrate activity by performing dot blot.
- Ensure buffers do not contain sodium azide, as it interferes with HRP activity.
- Extend signal exposure time.

Cat. No.	Product	Package
GTX16352	Trident 2X Tris Glycine SDS Sample Buffer	25 ml
GTX16354	Trident 2X Tris Glycine Native Sample Buffer	25 ml
GTX16357	Trident 6X Laemmli SDS Sample Buffer	25 ml
GTX50875	Trident Prestained Protein Ladder	500 µl
GTX16376	Trident Blue Prestained Protein Ladder	500 µl
GTX400006	Trident ECL plus	500 ml
GTX14698	Trident Sharp-ECL (femtogram)	100/200 ml
GTX49999	SignalPlus Antibody Enhancer (for Western Blot)	50 ml