Distorted Pattern

Western Blot Steps

01 SDS-PAGE Electrophoresis

02 Transfer

Band Patterns and Possible Reasons

a Uneven band sizes
- Poor gel polymerization
- Inappropriate running conditions

b Smiling effect on bands
- Inappropriate running conditions: too fast or too hot

c Blurry bands
- Electrophoresis voltage too high
- Incorrect running buffer

d Inadequate protein separation

Trapped air bubbles present during transfer

Possible Solution(s)

01 a
- Ensure that the APS and TEMED used for gel polymerization are properly added and mixed (and not expired)
- Ensure the gel did not dry out during storage
- Maintain similar salt concentrations of samples in all wells
- Load empty wells with 1X SDS-PAGE sample buffer

01 b
- Reduce voltage during electrophoresis
- Reduce gel running temperature by using chilled buffers or run the gel in the cold room

01 c
- Reduce voltage during electrophoresis
- Prepare fresh running buffer or using optimized running buffers from GeneTex

01 d
- Optimize gel percentage according to MW of target proteins (Large protein --> low gel percentage and vice versa)
- Recommended buffer systems:
  - For general use: Tris-Glycine buffer
  - For small-to-medium sized proteins: Bis-Tris buffer
  - For medium-to-large sized proteins: Bis-Tris buffer
  - For large sized proteins: Tris-Acetate buffer

02
- Remove air bubbles between the gel and membrane carefully before transfer
- Use optimized transfer buffers from GeneTex

Streaky Bands

Western Blot Steps

01 Sample Preparation

02 SDS-PAGE Electrophoresis

Possible Reasons

a Impurities in the sample

b Improper sample preparation

Protein overloaded

Possible Solution(s)

01 a
- Always spin the samples before loading and prevent loading of precipitates into wells

01 b
- Perform total protein or subcellular fraction preparation rapidly with an optimized extraction kit (e.g., Trident Membrane Protein Extraction Kit (GTX16373))

02
- Measure protein concentration properly
- Optimize loading amount to observe a specific signal

Black Dots

Western Blot Step

01 Blocking

Possible Reasons

Blocking reagents bind to antibodies

Possible Solution(s)

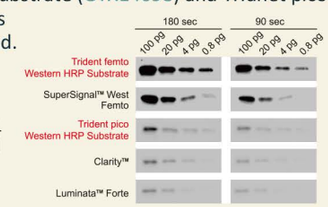
01
- Ensure blocking reagents (non-fat milk or BSA) are completely dissolved in the buffer
- Filter the blocking buffer if necessary
- Use an optimized blocking buffer system (e.g. Trident Universal Protein Blocking Reagent (animal serum free) (GTX30963))

White Bands

Western Blot Steps

	Possible Reasons	Possible Solution(s)
01 SDS-PAGE Electrophoresis	Excess protein loading	01 • Measure protein concentration properly • Optimize loading amount to observe a specific signal
02 Primary Antibody Secondary Antibody	Excess amount of antibodies	02 • Reduce the amount of antibody (Increase dilution factor)
03 ECL	Overexposure	03 • Shorten exposure time • Use digital imaging system to assess signal intensity over time

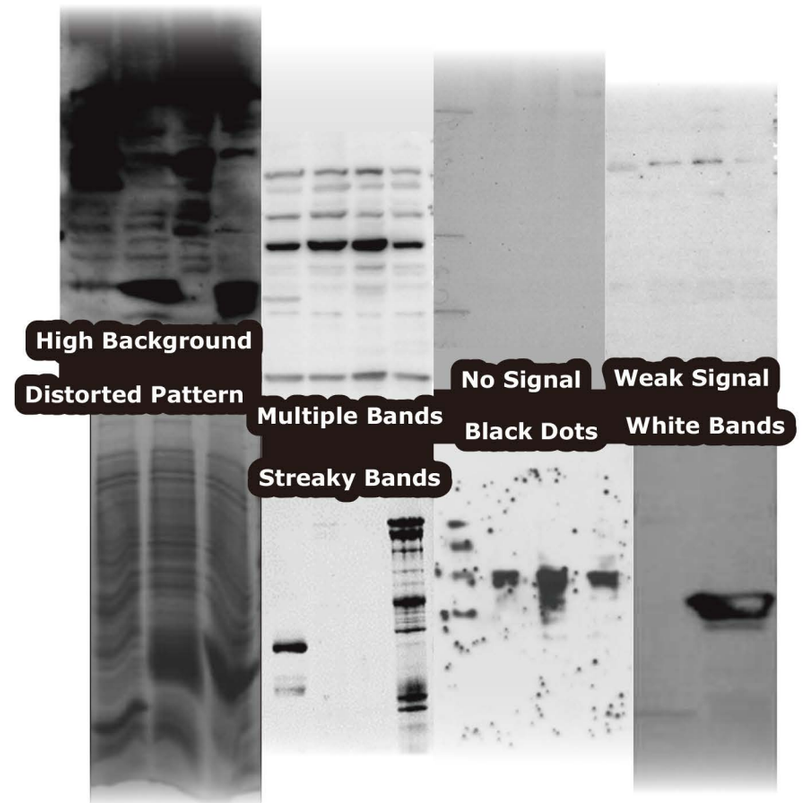
WB analysis using Trident femto Western HRP Substrate (GTX14698) and Tridnet pico Western HRP Substrate (GTX17435) with various sample amounts and exposure times as indicated.



* SuperSignal™ West Femto, Clarity ™, and Luminata ™ Forte are registered trademarks of Thermo Fisher Scientific, Bio-Rad, and EMD Millipore, respectively. The trademark holders are not affiliated with GeneTex and do not endorse the products described here.

GeneTex Your Expertise, Our Antibodies, Accelerated Discovery.

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.

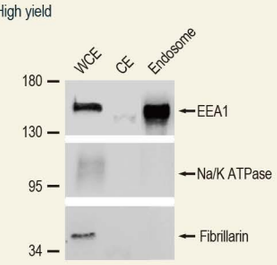
International Tel: 886.3.6208988
USA Tel: 1.949.553.1900 Toll-free: 1.877.436.3839

PD-W002-210705

High Background or Multiple Bands

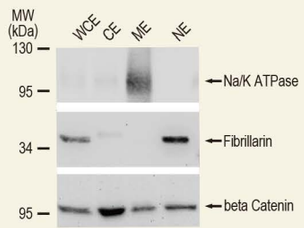
Trident Endosome Isolation Kit (GTX35192) is for rapid extraction and enrichment of endosome from cultured cells or tissues.

- Filter cartridge-based isolation
- No more density gradient ultracentrifugation
- Small amount of starting materials(~10^7cells or 10-20 mg tissue)
- Suitable for BOTH fresh or frozen tissue
- High yield



Trident Membrane protein extraction kit (GTX16373) is for rapid extraction of native total membrane proteins from cultured mammalian cells or tissues.

- Simple and user friendly
- Wide range of starting cells (1 - 50 millions / sample)
- Detergent and EDTA free
- No need for Dounce homogenizer or tissue blender
- Finish extraction in less than 45 min
- High yield



Possible Solution(s)

01 a
- Boil the samples properly to ensure appropriate protein denaturation
- Add dithiothreitol (DTT) or 2-mercaptoethanol (2-ME) freshly into the sample buffer for the reduction of disulfide bonds
- Check isoforms and/or alternative splicing information of the target protein

01 b
- Check post-translational modifications

02
- Use lysis buffers with protease inhibitors and phosphatase inhibitors
- Perform protein extraction on ice
- Aliquot samples and avoid repeated freeze-thaw cycles
- Perform protein extraction rapdly with an optimized extraction kit (e.g. Trident Total Protein Extraction Kit (GTX16372))

03
- Measure protein concentration properly
- Optimize loading amount to observe a specific sagnal

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

05
- Increase blocking duration
- Determine suitable blocking buffer (3-5% non-fat milk or BSA) or use an optimized blocking buffer system (e.g. Trident Universal Protein Blocking Reagent (animal serum free) (GTX30963))

06
- Check antibody datasheet for 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 the number or duration of washing steps
- Increase the concentration of detergents in the wash buffer
- Wash with 0.1% Tween 20-containing buffers (e.g., Trident 10X TBST (GTX30976))

08
- Decrease the concentration of secondary antibody
- Incubate with a secondary antibody only (without primary antibody) as a control

09
- Shorten 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	5/20 Tests
GTX35191	Trident Mitochondria Isolation Kit	5/20 Tests
GTX35192	Trident Endosome Isolation Kit	5/20 Tests
GTX30963	Trident Universal Protein Blocking Reagent (animal serum free)	100 ml
GTX48887	Trident PBS (tablets)	100 tablets

Check Solutions

Possible Reasons

Western Blot Steps

01 Protein Biology
- a Protein structure (multimerization or isoforms)
- b Protein post-translational modifications

02 Sample Preparation
- Protein degradation

03 SDS-PAGE Electrophoresis
- Excess protein loading

04 Transfer
- Improper membrane used

05 Blocking
- Inadequate blocking

06 Primary Antibody
- Non-specific binding of primary antibody

07 Wash
- Inadequate washing

08 Secondary Antibody
- Non-specific binding of secondary antibody

09 ECL
- Overexposure

Check Solutions

Possible Reasons

01 Protein Biology
- Low expression of protein of interest

02 Sample Preparation
- Protein degradation

03 SDS-PAGE Electrophoresis
- Insufficient protein loading

04 Transfer
- Incomplete or Over-transfer
  - Confirm transfer by staining membrane with Ponceau S, Amido Black or India Ink
  - Assess successful protein transfer by using prestained protein markers (see GTX50875 and GTX16376)

05 Blocking
- Over-blocking

06 Primary Antibody
- Insufficient primary antibody

07 Wash
- Excessive washing

08 Secondary Antibody
- a Incorrect secondary antibody used
- b Insufficient secondary antibody

09 ECL
- Insufficient signal development

Weak or No Signal

Possible Solution(s)

01
- Increase the loading amount of protein samples
- Induce target protein expression via stimulation
- Enrich target protein abundance using specific subcellular fractionations (e.g. nuclear fraction (GTX16374) or membrane fraction (GTX16373))
- Enrich target protein abundance by immunoprecipitation
- Check antibody datasheet and run a recommended positive control sample

02
- Use lysis buffers with protease inhibitors and phosphatase inhibitors
- Perform protein extraction on ice
- Aliquot samples and avoid repeated freeze-thaw cycles
- Perform protein extraction rapdly with an optimized extraction kit (e.g. Trident Total Protein Extraction Kit (GTX16372))

03
- Measure protein concentration properly
- Load at least 20 - 30 µg protein per well
- Ensure approapriate sample buffer and runnning buffer is used

04
- PVDF membrane: activate membrane with methanol before transfer
- Nitrocellulose membrane: pre-soak membrane in transfer buffer before transfer
- Adjust transfer time according to the size of the target protein (The larger the protein, the longer time is needed for transfer.)
- Avoid over-transfer of small proteins by reducing transfer voltage or time

05
- Adjust blocking time (1-3 hours at room temperature)
- Reduce blocking reagent concentration
- Use an optimized blocking buffer system (e.g. Trident Universal Protein Blocking Reagent (animal serum free) (GTX30963))

06
- Check antibody datasheet for recommended dilutions
- Increase antibody concentration if target protein is less abundant
- Extend incubation time to overnight at 4°C
- Check antibody expiring date
- Avoid re-use primary antibody
- Use signal enhencer kit (e.g. SignalPlus Antibody Enhancer -for Western Blot(GTX49999))

07
- Decrease the number or duration of washing steps

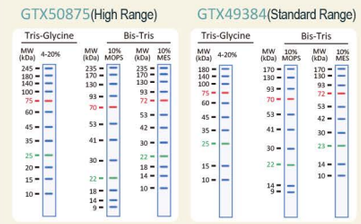
08 a
- Use secondary antibody directed against the specific host species and immunoglobulin type for the primary antibody

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

09
- Make sure the HRP substrate reagents have not expired
- Confirm substrate activity by performing dot blot
- Ensure no sodium azide(NaN_3) in the antibody dilution buffer, as it interferes HRP activity
- Extend signal exposure time
- Use HRP substrate reagents with higher sensitivity (e.g. Trident pico Western HRP Substrate (GTX17435) or Trident femto Western HRP Substrate (GTX14698))

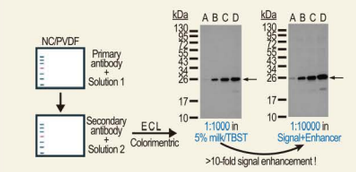
Trident Prestained Protein Ladder (Standard Range: GTX49384) (High Range: GTX50875)

- Ready to use
- 2~5 µL per well for general western blots
- 3-color protein standard with 10 (GTX49384) or 12 (GTX50875) pre-stained proteins
- Blue bands with 1 green (25 kDa) and 1 red (75 kDa) band
- Cover wide range molecular weights from 10 to 245 kDa
- Compatible with multiple buffer systems: Tris, MOPS, MES
- Compatible with PVDF, nylon, and nitrocellulose 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



Cat. No.	Product	Package
GTX50875	Trident Prestained Protein Ladder (High Range)	500 µl
GTX49384	Trident Prestained Protein Ladder (Standard Range)	500 µl
GTX16376	Trident Blue Prestained Protein Ladder	500 µl
GTX400006	Trident plus Western HRP Substrate	500 ml
GTX17435	Trident pico Western HRP Substrate	100ml/500ml
GTX14698	Trident femto Western HRP Substrate	100/200 ml
GTX49999	SignalPlus Antibody Enhancer (for Western Blot)	50 ml