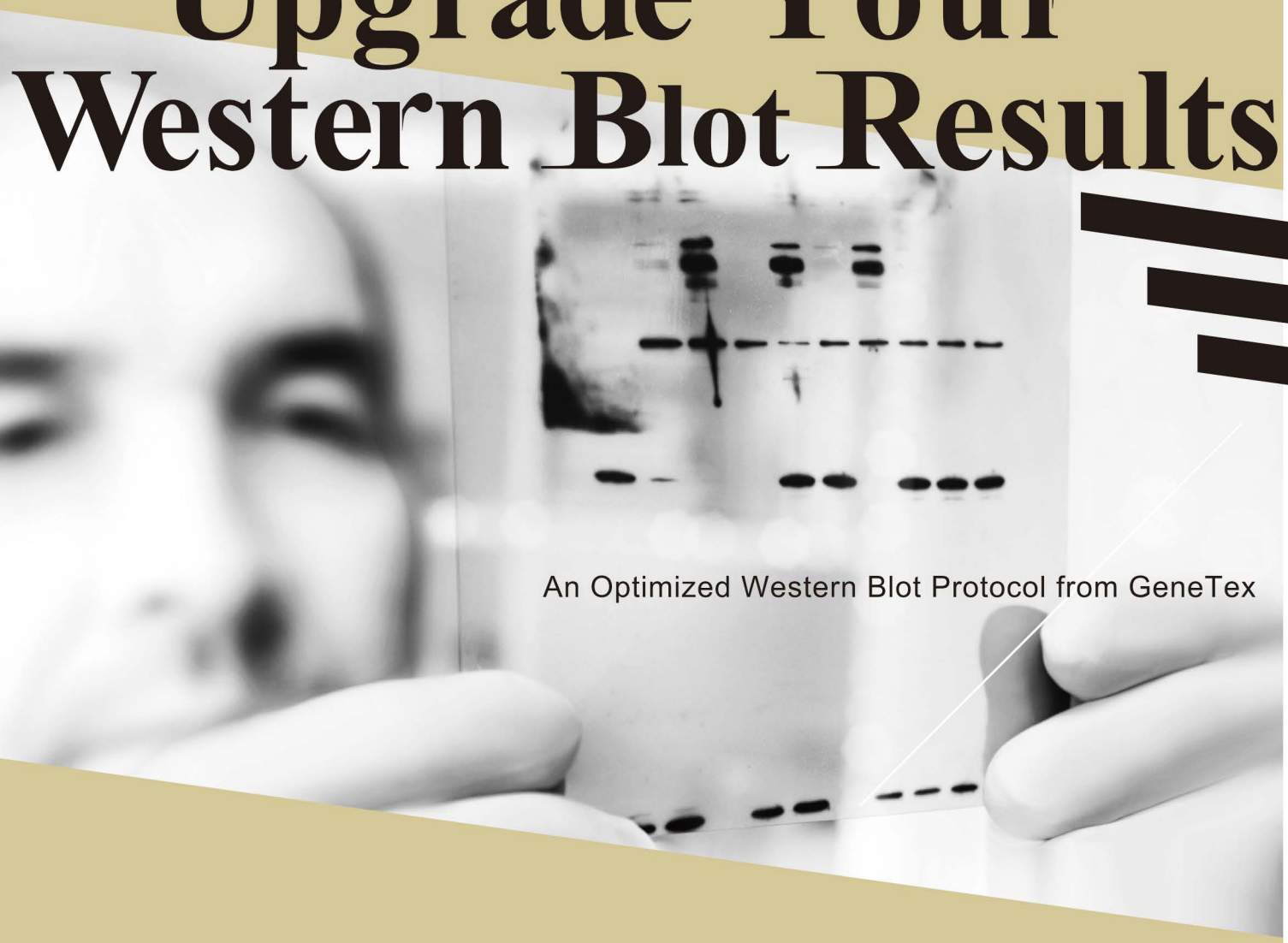
GeneTex

Your Expertise, Our Antibodies, Accelerated Discovery.

Upgrade Your Western Blot Results



An Optimized Western Blot Protocol from GeneTex

TRIDENT

make blots better.

Preparation of Protein Extracts

1. Prepare extracts from cultured cells or tissues with our **Trident Extraction Kits**.

> The total number of cells per ml and the cell equivalent loaded per lane of gel should be optimized specifically for each protein and antibody.

2. Determine the protein concentration of the extract and transfer the appropriate amount of your sample to a new tube. Aliquot and freeze the stock at -20℃ or below.

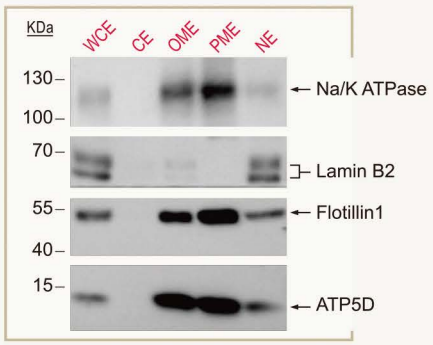
3. Add **Trident Sample Buffer** to your sample and boil at 100℃ for 5 minutes to denature the proteins. Spin the sample briefly and load onto your SDS-PAGE gel.

> Add Dithiothreitol (DTT) or β-mercaptoethanol (2-ME) to the Trident Sample Buffer before use to reduce proteins, if necessary.

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
GTX16352	Trident 2X Tris-Glycine SDS Sample Buffer	25 ml
GTX16354	Trident 2X Tris-Glycine Native Sample Buffer	25 ml
GTX16355	Trident 4X Laemmli SDS Sample Buffer	25 ml
GTX16357	Trident 6X Laemmli SDS Sample Buffer	25 ml

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 min
- ✓ High yield



SDS-PAGE and Gel Transfer

1. Load 30 µg of each protein extract or 100 ng of purified protein into the wells of the SDS-PAGE gel. Load an appropriate amount of **Trident Blue Prestained Protein Ladder** or **Trident Prestained Protein Ladder** to one or more additional lanes.

2. Run the gel in 1X **Trident Running Buffer** for 1-2 hours at 50-100 V.

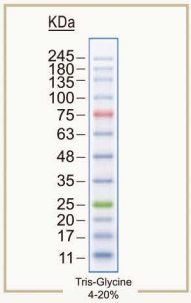
> We recommend setting the electrophoresis at a lower voltage and for a longer time. This should result in clearer bands and better resolution.

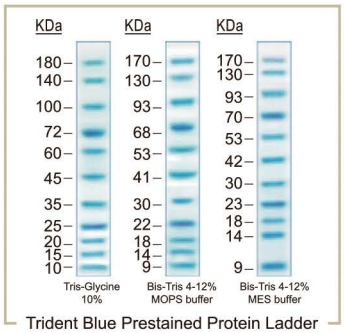
3. Transfer the proteins from the gel to a nitrocellulose or methanol-rinsed PVDF membrane in 1X **Trident Transfer Buffer**.

> Optional: Confirm successful protein transfer by Ponceau Red staining before proceeding to the next step.

Cat. No.	Product	Package
GTX16362	Trident 1M Tris-HCl, pH6.8	1 L
GTX16364	Trident 1M Tris-HCl, pH7.4	1 L
GTX16365	Trident 1M Tris-HCl, pH8.8	1 L
GTX16368	Trident 0.5 M EDTA, pH8.0	500 ml
GTX16370	Trident 20% SDS (w/v)	500 ml
GTX16338	Trident 10X Tris-Glycine-SDS Running Buffer	1 L
GTX16340	Trident 10X Tris-Glycine Native Running Buffer	1 L
GTX16342	Trident 20X MOPS-SDS Running Buffer	1 L
GTX16343	Trident 20X MES-SDS Running Buffer	1 L
GTX16344	Trident 20X Tris-Acetate-SDS Running Buffer	1 L
GTX16345	Trident 20X Tris-HEPES-SDS Running Buffer	1 L
GTX16346	Trident 10X Tris-Glycine Transfer Buffer	1 L
GTX16347	Trident 25X Tris-Glycine Transfer Buffer	1 L
GTX16348	Trident 20X Tris-Bicine Transfer Buffer	1 L
GTX16358	Trident 10X Multi-Western Stripping Buffer	100 ml

The Trident Prestained Protein Ladder (GTX50875) is a three-color protein standard with 12 prestained proteins covering a wide range of molecular weights from 10kDa to 245 kDa. Proteins are covalently coupled with a blue chromophore except for two reference bands (one green band at 25 kDa and one red band at 75 kDa).





Trident Blue Prestained Protein Ladder
(GTX16376)

Cat. No.	Product	Package
GTX50875	Trident Prestained Protein Ladder	500 µl
GTX16376	Trident Blue Prestained Protein Ladder	500 µl

SCAN FOR ONLINE PRODUCT DETAILS!

Blocking, Antibody Incubation, and Washing

1. Blocking: Incubate the blot in 3% non-fat milk / PBST or **Trident Universal Protein Blocking Reagent** for 30-60 minutes at RT.

2. Primary antibody incubation: Incubate the blot in 1 % non-fat milk / PBST or **Trident Universal Protein Blocking Reagent** containing the primary antibody at the proper dilution for two hours at RT or 4℃ overnight.

3. Washing: Wash the blot with 1X PBST for 10 minutes once and for 5 minutes twice.

4. Secondary antibody incubation: Incubate the blot in 1 % non-fat milk / PBST or **Trident Universal Protein Blocking Reagent** containing the HRP-conjugated secondary antibody at the proper dilution for one hour at RT.

5. Washing: Wash the blot with 1 X PBST three times, each for 10 minutes.

The Trident Universal Protein Blocking Reagent (animal serum-free) (GTX30963) does not contain any animal serum, and can be used for all IHC and ICC kits, ELISAs, and WBs.

Cat. No.	Product	Package
GTX30963	Trident Universal Protein Blocking Reagent (animal serum-free)	100 ml
GTX30977	Trident 10X PBST	100 ml
GTX30976	Trident 10X TBST	100 ml
GTX48887	Trident PBS (tablets)	100 tablets
GTX48886	Trident TBS (tablets)	100 tablets

ECL-based Signal Detection

Follow the instructions of the **Trident ECL plus (GTX400006)** or **Trident Sharp-ECL (GTX14698)** for detection of your signal.

Cat. No.	Product	Package
GTX400006	Trident ECL plus	500 ml
GTX14698	Trident Sharp-ECL (femtogram)	100/200 ml

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