Quality Antibodies · Quality Results

GeneTex

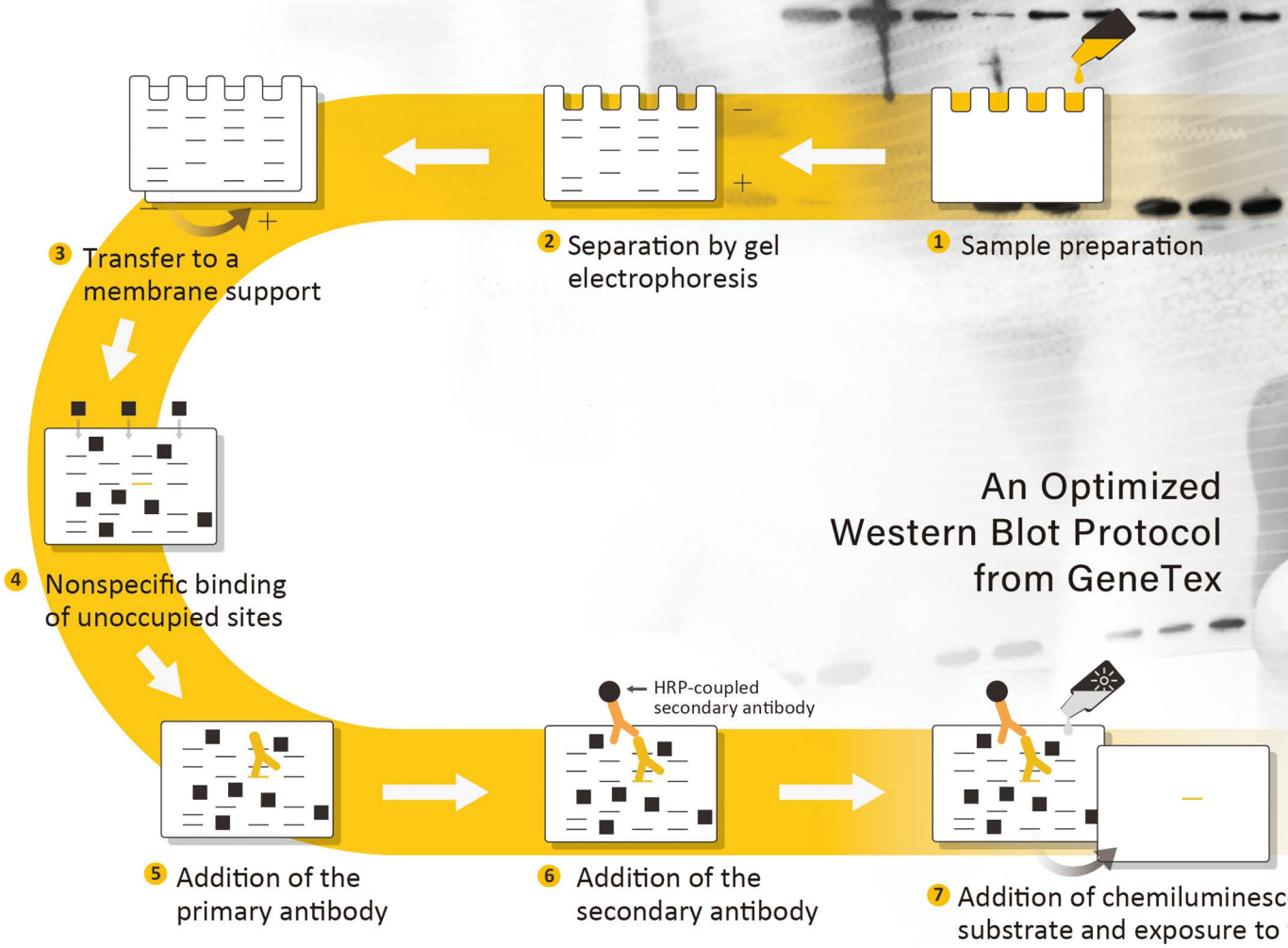
Upgrade Your Western Blot Results

Your Expertise

Our Antibodies

Accelerated Discovery

www.genetex.com



An Optimized Western Blot Protocol from GeneTex

TRIDENT

make blots better.

www.GeneTex.com

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.

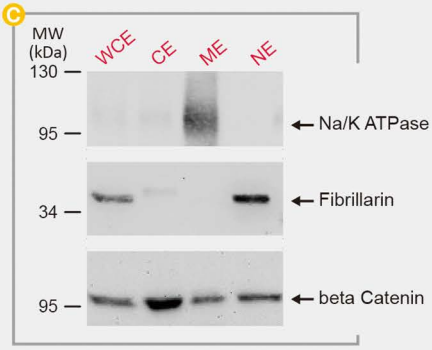
> Add Dithiothreitol (DTT) or β-mercaptoethanol (2-ME) to the Trident Sample Buffer before use to reduce proteins, if necessary.

Orthogonal Validation Citation Support

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

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)
- Detergent- and EDTA-free
- No need for Dounce homogenizer or tissue blender
- Finish extraction in less than 45 minutes
- High yield



WCE : Whole Cell Extract CE : Cytosolic Extract
ME : Membrane Extract NE : Nuclear Extract

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 (GTX16376) or Trident Prestained Protein Ladder (High Range: GTX50875 or Standard Range: GTX49384) 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.

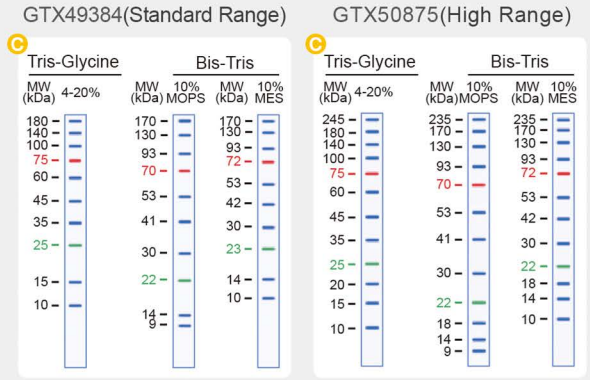
Optional: Confirm successful protein transfer by Ponceau Red staining before proceeding to the next step.

Citation Support

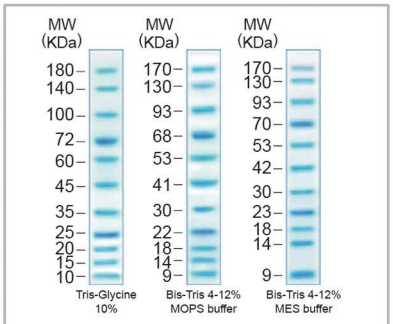
Cat. No.	Product	Package
GTX16364	Trident 1M Tris-HCl, pH7.4	1 L
GTX16368	Trident 0.5 M EDTA, pH8.0	500 ml
GTX16370	Trident 20% SDS (w/v)	500 ml
GTX16358	Trident 10X Multi-Western Stripping Buffer	100 ml

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

- Ready to use
- 2~5µL 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



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



Trident Blue Prestained Protein Ladder (GTX16376)

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 (GTX30963) 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 (GTX30963) 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 (GTX30963) 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 WB, ELISA, IHC and ICC/IF experiments.

Citation Support

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

ECL-based Signal Detection

Follow the instructions of the Trident plus Western HRP Substrate (GTX400006) or Trident femto Western HRP Substrate (GTX14698) for detection of your signal.

Citation Support

Cat. No.	Product	Package
GTX400006	Trident plus Western HRP Substrate	500 ml
GTX17435	Trident pico Western HRP Substrate	100/500ml
GTX14698	Trident femto Western HRP Substrate	100/200 ml

WB analysis using Trident femto Western HRP Substrate (GTX14698) with varied sample amount and exposure times.

Serial dilutions of purified mCherry recombinant protein were prepared and applied to WB assay. Identical blots were incubated with 1 ml of Trident femto Western HRP Substrate (GTX14698), SuperSignal™West Femto, Clarity™, and Luminata™ Forte.

* The trademark holders are not affiliated with GeneTex and do not endorse the products described here.

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