

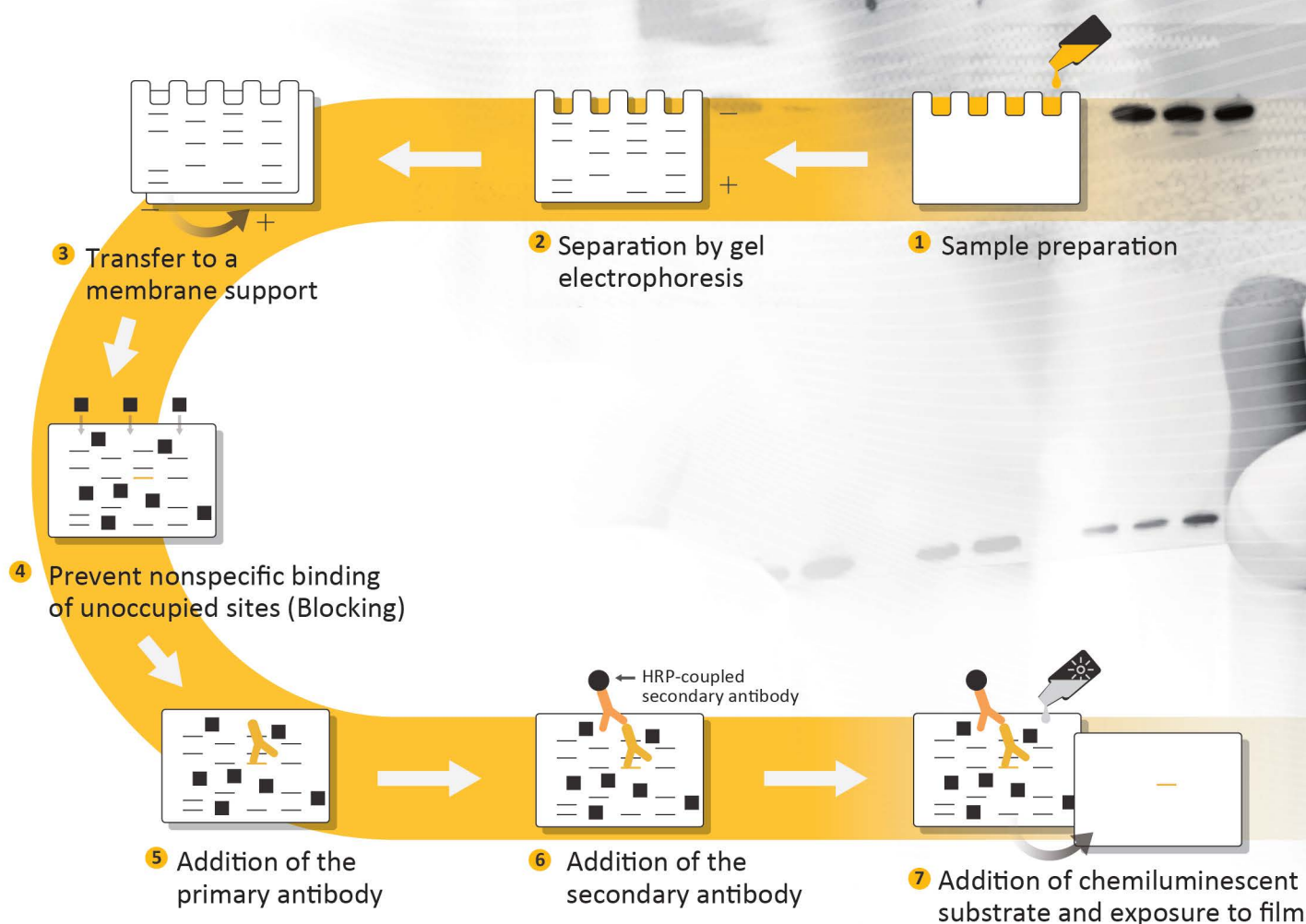
# Upgrade Your Western Blot Results

Your Expertise

Our Antibodies

Accelerated Discovery

[www.genetex.com](http://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°C or below.

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



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

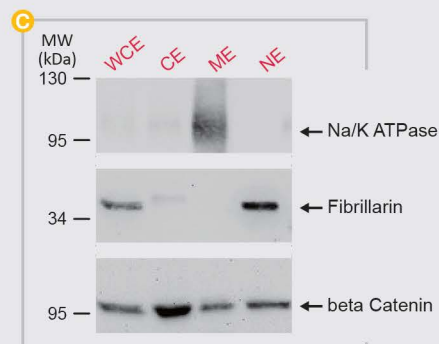
Orthogonal Validation

Citation Support

| Cat. No.  | Product                                               | Package    |
|-----------|-------------------------------------------------------|------------|
| GTX400005 | Trident RIPA Lysis Buffer                             | 100 ml     |
| GTX400033 | Trident RIPA Lysis Buffer (Low SDS)                   | 250 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 |
| GTX00835  | Trident High-Efficiency Exosome Precipitation Reagent | 20 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)
- 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 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 Transfer Buffer.



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

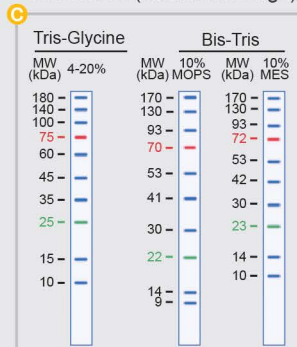
**C** Citation Support

| Cat. No.          | Product                                            | Package |
|-------------------|----------------------------------------------------|---------|
| GTX16370          | Trident 20% SDS (w/v)                              | 500 ml  |
| <b>C</b> GTX50875 | Trident Prestained Protein Ladder (High Range)     | 500 µl  |
| <b>C</b> GTX49384 | Trident Prestained Protein Ladder (Standard Range) | 500 µl  |
| GTX16376          | Trident Blue Prestained Protein Ladder             | 500 µl  |

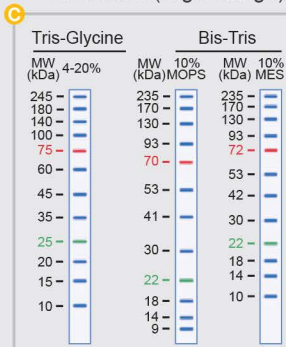
The Trident Prestained Protein Ladder  
(Standard Range: GTX49384)  
(High Range: GTX50875)

- 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

**C** GTX49384 (Standard Range)



**C** GTX50875 (High Range)



## 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°C 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.

| 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        |
| Ⓢ GTX213110-01 | Goat Anti-Rabbit IgG antibody (HRP)                            | 1 ml          |
| Ⓢ GTX213111-01 | Goat Anti-Mouse IgG antibody (HRP)                             | 1 ml          |
| Ⓢ GTX224125-01 | Rabbit Anti-Rat IgG antibody (HRP)                             | 1 ml          |
| Ⓢ GTX224126-01 | Rabbit Anti-Chicken IgY antibody (HRP)                         | 1 ml          |
| Ⓢ GTX232040-01 | Donkey Anti-Goat IgG antibody (HRP)                            | 1 ml          |
| Ⓢ GTX221666-01 | EasyBlot anti Rabbit IgG (HRP)                                 | 250 µl, 50 µl |
| Ⓢ GTX221667-01 | EasyBlot anti Mouse IgG (HRP)                                  | 250 µl, 50 µl |
| Ⓢ GTX628474-01 | EasyBlot anti Rat IgG (HRP)                                    | 250 µl, 50 µl |
| Ⓢ GTX628547-01 | EasyBlot anti Goat IgG (HRP)                                   | 250 µl, 50 µl |
| Ⓢ GTX628906-01 | EasyBlot anti Sheep IgG (HRP)                                  | 250 µl, 50 µl |

### EasyBlot®: The easiest way to get the best result!

#### Clear

Efficiently eliminates denatured IgG masking of your target protein's signal

#### Compatible

Suitable for WB following either IP or co-IP analysis using Protein A-, Protein G-, or Agarose-conjugated antibodies

#### Comprehensive

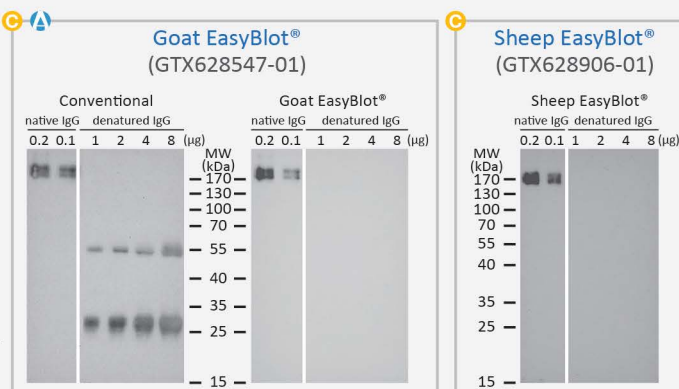
Broad range of EasyBlot® reagents for detecting various hosts: rabbit, mouse, rat, goat or sheep

#### Convenient: Simple and user-friendly!

Easy to switch from regular secondary antibodies to EasyBlot® secondary antibodies

#### Combine with EasyBlocker to further optimize your specific signal

Significantly reduce background generated by free Protein A/G with the EasyBlocker reagent



## 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.

| Cat. No.    | Product                             | Package    |
|-------------|-------------------------------------|------------|
| Ⓢ GTX400006 | Trident plus Western HRP Substrate  | 500 ml     |
| Ⓢ GTX17435  | Trident pico Western HRP Substrate  | 100/500 ml |
| Ⓢ 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.

