

# Protocol: Western Blot

## MATERIALS/ REAGENTS/ BUFFERS

- Tris Glycine SDS Sample Buffer
- Tris-Glycine-SDS Running Buffer
- Tris Glycine Transfer Buffer
- Nitrocellulose membrane or Polyvinylidene fluoride membrane (PVDF)
- ECL<sup>®</sup> reagents (GTX400006)
- PBST (GTX30977)
- Blocking Buffer (GTX30963)
- Prestained Protein Ladder (GTX50875)

## INSTRUMENT

- Electrophoresis apparatus
- Electronic blotting apparatus

## PROTOCOL

### 1. Preparation of Protein Extract

- 1.1 Prepare the protein extract in sample buffer and boil at 100°C for 5 minutes (min) to denature the proteins.  
Note: Add Dithiothreitol (DTT) or  $\beta$ -mercaptoethanol (2-ME) to reduce proteins, if necessary.

### 2. SDS-PAGE and Gel Transfer

- 2.1 Load samples into the wells of the SDS-PAGE gel. Load an appropriate amount of pre-stained protein ladder (GTX50875) to one or more additional lanes.
- 2.2 Run the gel in 1X running buffer for 1-2 hours at 50-100 V.
- 2.3 Transfer the proteins from the gel to a nitrocellulose or methanol-rinsed PVDF membrane in 1X transfer buffer

### 3. Blocking, Antibody Incubation, and Washing

- 3.1 Blocking: Incubate the blot in 3% non-fat milk/ PBST or the universal protein blocking reagent (GTX30963) for 30-60 min at RT.
- 3.2 Primary antibody incubation: Incubate the blot in 1% non-fat milk / PBST or the universal protein blocking reagent (GTX30963) containing the primary antibody at the proper dilution for two hours at RT or 4°C overnight.
- 3.3 Washing: Wash the blot with 1X PBST for 10 min once and for 5 min twice.
- 3.4 Secondary antibody incubation: Incubate the blot in 1% non-fat milk / PBST or the universal protein blocking reagent (GTX30963) containing the HRP-conjugated secondary antibody at the proper dilution for one hour at RT.
- 3.5 Washing: Wash the blot with 1 X PBST three times, each for 10 min.

### 4. ECL-based Signal Detection

- 4.1 Follow the instructions of the Trident ECL plus (GTX400006) for detection of signals.

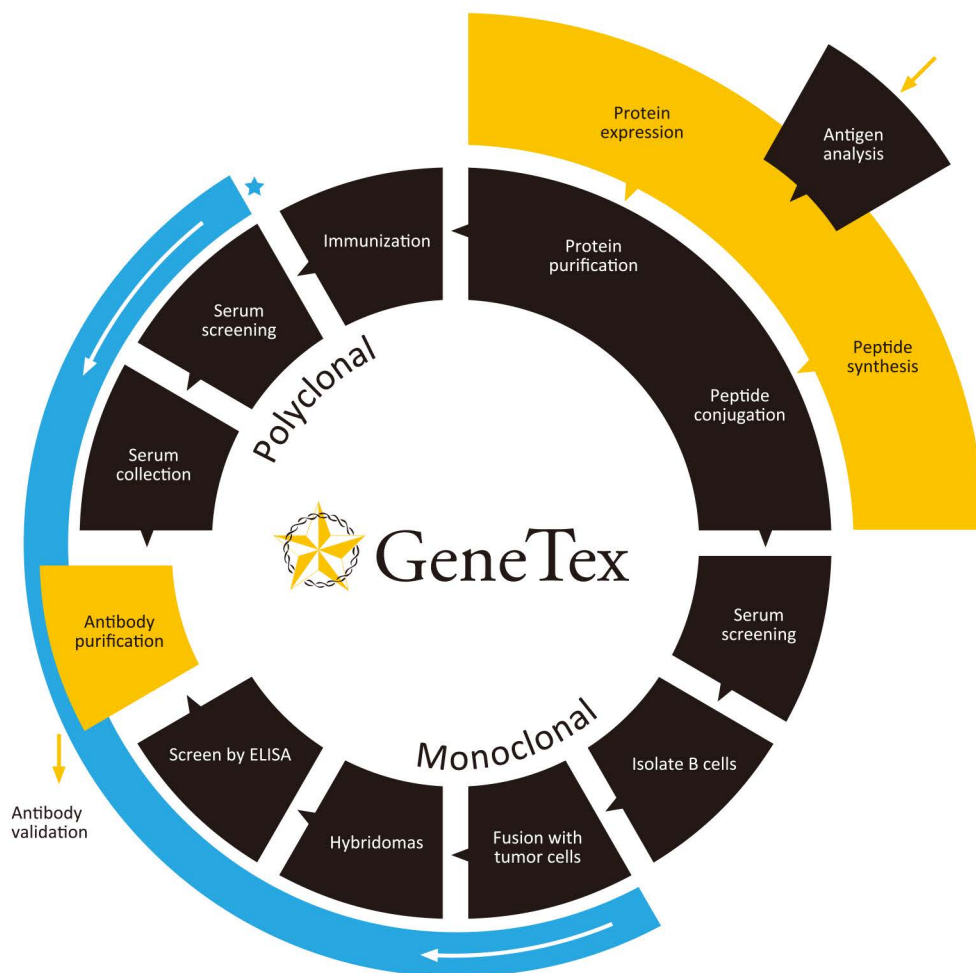


# About GeneTex

GeneTex started as a small scientist-founded company in San Antonio, Texas, and has grown to become one of the top antibody manufacturers.

Our corporate mission, to provide our customers with quality reagents and to accelerate life sciences research, reflects the philosophy and approach we employ when we manufacture our products. Through extensive research, development, and quality-assurance testing, we have produced and validated a comprehensive collection of antibodies and research reagents.

Due to GeneTex's experience in antibody production, we are uniquely capable of providing technical support and troubleshooting assistance for our customers.



★ Recombinant Antibody Generation



# Selection you need

## Comprehensive Coverage

Genetex currently offers more than **55,000** products including over **45,000** primary antibodies that cover eleven different research areas. We continue to build upon our product portfolio to ensure that novel targets are available to researchers and that Genetex keeps pace with new scientific discovery.

In addition to our comprehensive catalog of primary antibodies, GeneTex also offers a wide variety of quality reagents, kits and services.

