

# Protocol: Dot Blot

Note: Dot blot is a simple technique to detect proteins. It is a quick assay that is different from a western blot because it does not separate the proteins via electrophoresis. Sample proteins are spotted on a membrane and hybridized with an antibody that acts as a probe. Dot blot results give semi-quantitative measurements of the spotted proteins.

## MATERIAL

- Primary and HRP-conjugated secondary antibodies
- Nitrocellulose membrane
- ECL solution
- Plastic film wrap
- X-Ray film

## BUFFERS

- TBS: 20mM Tris-HCl, 150mM NaCl, pH 7.5
- TBS-T: 0.05% Tween20 in TBS
- Blocking solution: 5%BSA in TBS-T

## METHODS

1. Label nitrocellulose membrane, spot slowly 2 $\mu$ l of sample on the membrane (3-4 mm diameter) and allow to dry.
2. Incubate the membrane in blocking solution for 1 hr at RT.
3. Incubate with primary antibody (in TBS-T with 0.1% BSA) for 30 min at RT.
4. Wash the membrane 3X (5 min each) with TBS-T.
5. Incubate the membrane with the HRP-conjugated secondary antibody for 30 min at RT.
6. Wash the membrane 3X (10 min each) with TBS-T.
7. Incubate the membrane with the ECL solution for 1 min. Apply plastic film wrap and expose to x-ray film at different exposure times. Compare samples with standard to estimate concentration.

# ABOUT



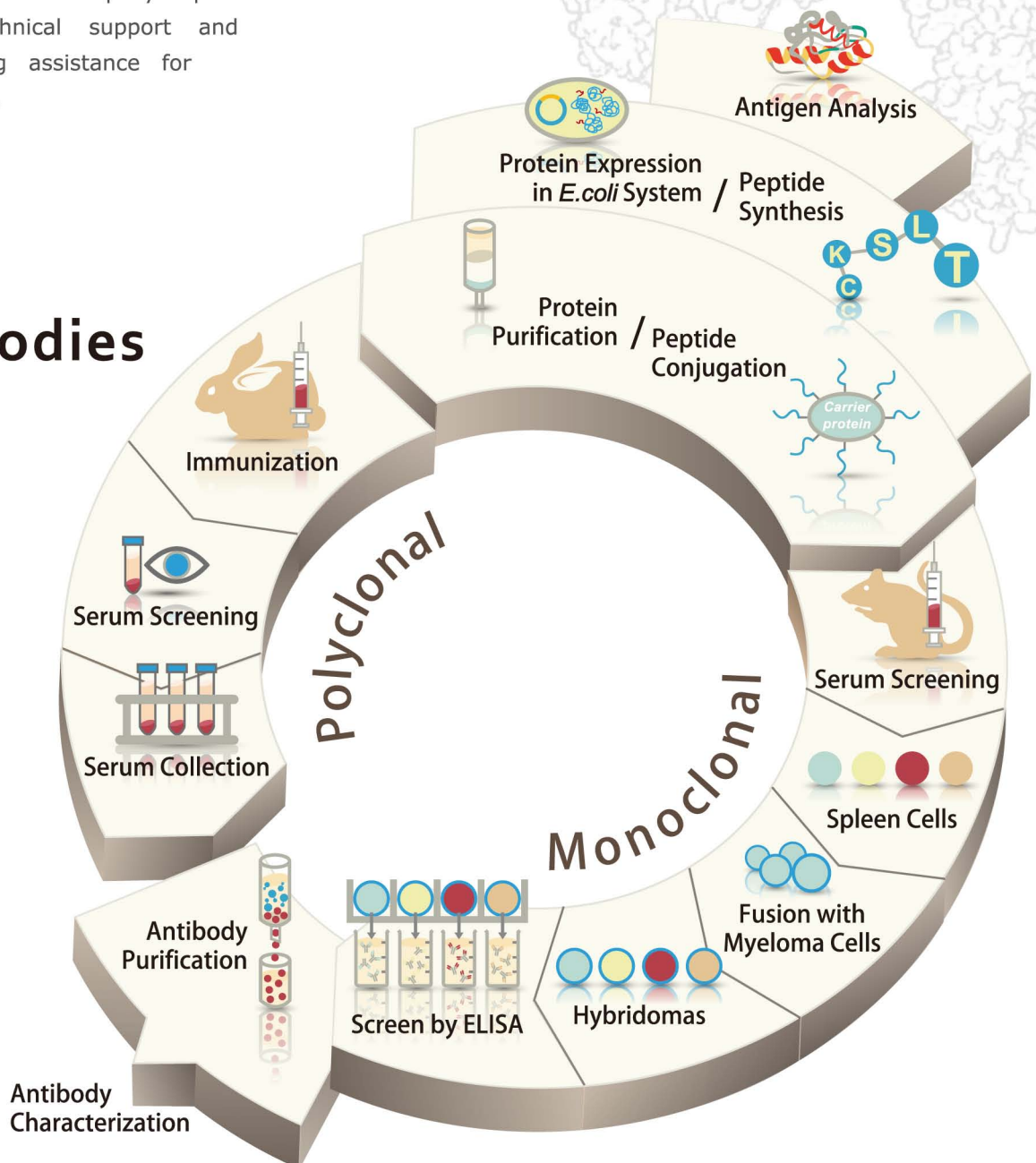
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Starting as a small scientist-founded company in San Antonio, Texas, GeneTex has grown to become one of the top antibody manufacturers and has solidified its position in the life sciences industry.

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.

## Our Antibodies



# 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.

