Protocol: Indirect ELISA

Note :

The Enzyme-linked Immunosorbent Assay (ELISA) combines the specificity of antibodies with the sensitivity of simple enzyme assays. Indirect ELISA uses antigens coupled to an easily-assayed enzyme. It can be used to measure concentration of antibody from unpurified sample.

MATERIALS/ REAGENTS/ BUFFERS

- Antigen (protein or carrier conjugated peptide)
- Primary antibody or sample containing antibody
- HRP-conjugated secondary antibody
- High binding 96-well microtiter plate (NUNC maxisorb flat bottom microtiter plate or Costar stripwellTM plate)
- Coating buffer:
  0.1M Bicarbonate/carbonate buffer pH9.6 or PBS pH7.4
- Blocking solution:
  Commonly used blocking agents are 1% BSA , 5% serum, 5% non-fat dry milk, 2.5% casein or 1% gelatin in PBS.
- Wash solution:
  PBS or TBS (pH 7.4) with detergent 0.05% (v/v) Tween20
- TMB substrate:
  Prepare substrate solution according to manufacturer's instruction
- Stop solution:
  1M H_3PO_4 or 2N H_2SO_4

INSTRUMENT

ELISA plate washer (optional)

ELISA plate reader

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PROTOCOL

1. Dilute antigen to a final concentration of 1-10 µg/ml in coating buffer (PBS or carbonate buffer). To coat the plate, add 100 µl of diluted antigen into wells. Concentration of antigen can be optimized.
2. Cover plates with adhesive plastic film and incubate for 4 hours at room temperature or 4°C overnight. Optimize coating incubation time when necessary.
3. Empty the wells and wash 3X with 300 µl /well of wash solution. Remove remaining drops by patting the plate on an absorbent paper after final wash.
4. Block non-specific binding by adding 200 µl blocking solution to each well.
5. Cover plates with adhesive plastic film and incubate for at least 1 hr at room temperature or overnight at 4°C.
6. (Optional) If not to be used immediately after blocking, sealed the plate and freeze at -70°C until use.
7. Empty and wash plates 2X with wash solution as previously done in step 3.
   Add 100 µl of diluted sample (antibody) to each well. Dilute sample (antibody) with blocking buffer when necessary. For accurate quantitative results, use samples of known concentration to make standard curve and compare the signal of unknown samples against those of a standard curve. Standards (duplicates or triplicates) and blank must be run on the same plate as sample to ensure accuracy.
8. Cover the plate with adhesive plastic film and incubate for 1 hour at room temperature or overnight at 4°C if ELISA signal is weak.
9. Repeat washes 3X as in step 3.
10. Dilute HRP-conjugated secondary antibody to optimal concentration according to the manufacturer in blocking solution immediately before use. Do not
11. include azide in the buffers as it inactivates HRP. Add 100 µl of diluted HRP-conjugated secondary antibody to each well.
12. Cover the plate with adhesive plastic film and incubate for 1 to 2 hours at room temperature.
13. Repeat washes 5X as in step 3.
14. Add 100 µl TMB solution to each well, incubate for 15-30 min at room temperature.
15. Add 100 µl stopping solution
16. Read optical density at 450 nm on an ELISA plate reader.
17. Analysis of data for quantitative measurement:
    Prepare a standard curve from the data of serial dilutions of control antibody with concentration at X-axis and absorbency at Y-axis. Interpolate the concentration of the sample from this standard curve.

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ABOUT

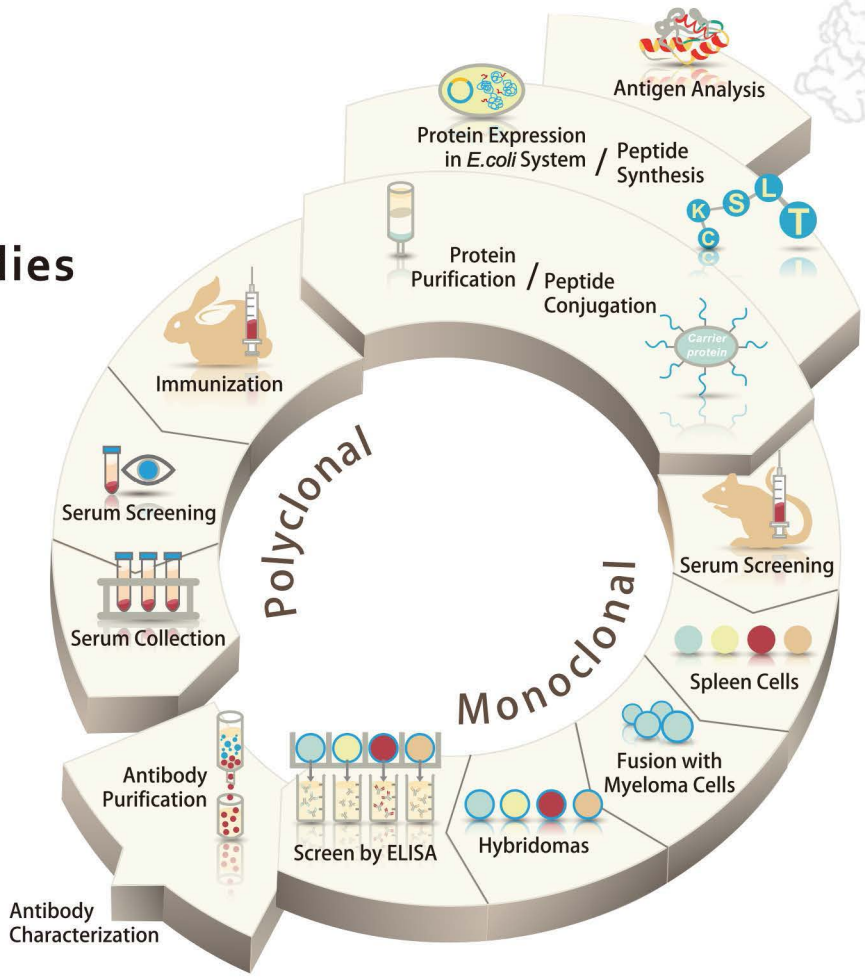
GeneTex

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.

Our Antibodies



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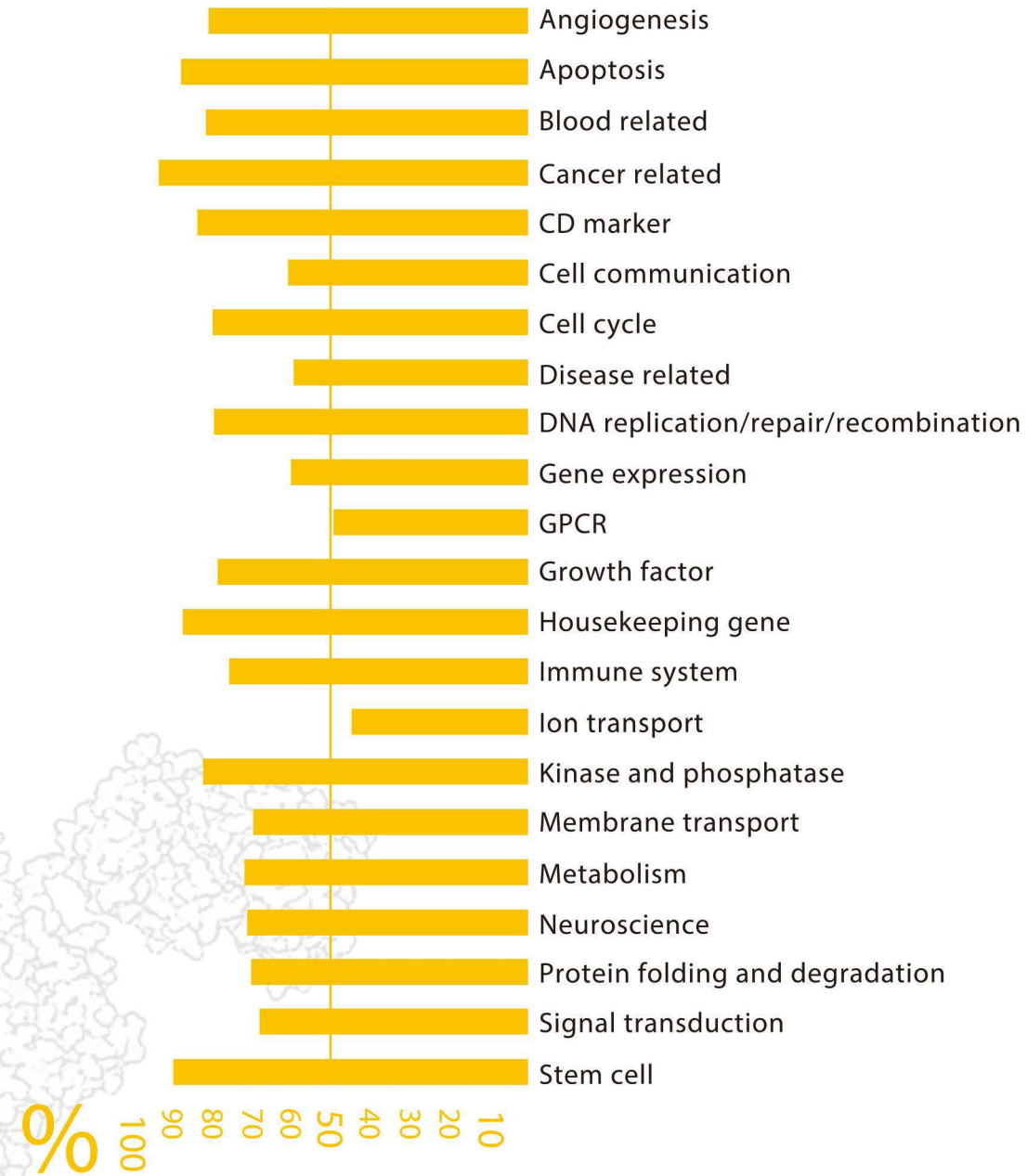

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