

Protocol: Sandwich ELISA

Note :

The Enzyme-linked Immunosorbent Assay (ELISA) combines the specificity of antibodies with the sensitivity of simple enzyme assays. Sandwich ELISA measures the amount of antigen between two layers of antibodies (capture and detection antibody). It provides a useful measurement of antigen concentration in unpurified samples.

Sandwich ELISA procedures can be difficult to optimize and paired antibodies are needed. Therefore, use antibodies only when they have been specifically tested for sandwich ELISA.

MATERIALS/ BUFFERS/ REAGENTS

- Antigen (protein or carrier conjugated peptide)
- Primary capture and detection antibody
- HRP-conjugated secondary antibody
- High binding 96-well microtiter plate (NUNC maxisorb flat bottom microtiter plate or Costar stripwell™ 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 H₃PO₄ or 2N H₂SO₄

INSTRUMENT

ELISA plate washer (optional)

ELISA plate reader

PROTOCOL

1. Dilute primary capture antibody to a final concentration of 1-10 ug/ml in coating buffer (PBS or carbonate buffer). To coat the plate, add 100 µl of diluted antibody into wells. Concentration of antibody 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.

PROTOCOL

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.
8. Add 100 µl of appropriately diluted samples (antigens) to each well. Dilute sample in blocking solution. 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.
9. Cover plates with adhesive plastic film and incubate for 2 hr at room temperature or overnight at 4°C if ELISA signal is weak.
10. Empty and wash plates 3X with wash solution as previously done in step 3.
11. Add 100 µl of diluted primary detection antibody to each well. Dilute antibody with blocking buffer according to the manufacturer.
12. Cover the plate with adhesive plastic film and incubate for 1 to 2 hours at room temperature. If detection antibody was HRP conjugated, skip to step 16.
13. Empty and wash plates 3X with wash solution as previously done in step 3
14. Dilute HRP-conjugated secondary antibody to optimal concentration according to the manufacturer in blocking solution immediately before use. Do not include azide in the buffers as it inactivates HRP. Add 100 µl of diluted HRP-conjugated secondary antibody to each well.
15. Cover the plate with adhesive plastic film and incubate for 1 to 2 hours at room temperature.
16. Empty and wash plates 5X with wash solution as previously done in step 3
17. Add 100 µl TMB solution to each well, incubate for 15-30 min at room temperature.
18. Add 100 µl stopping solution
19. Read optical density at 450 nm on an ELISA plate reader.
20. Analysis of data for quantitative measurement:
Prepare a standard curve from the data of serial dilutions of antigen with concentration at X-axis and absorbency at Y-axis. Interpolate the concentration of the sample from this standard curve.



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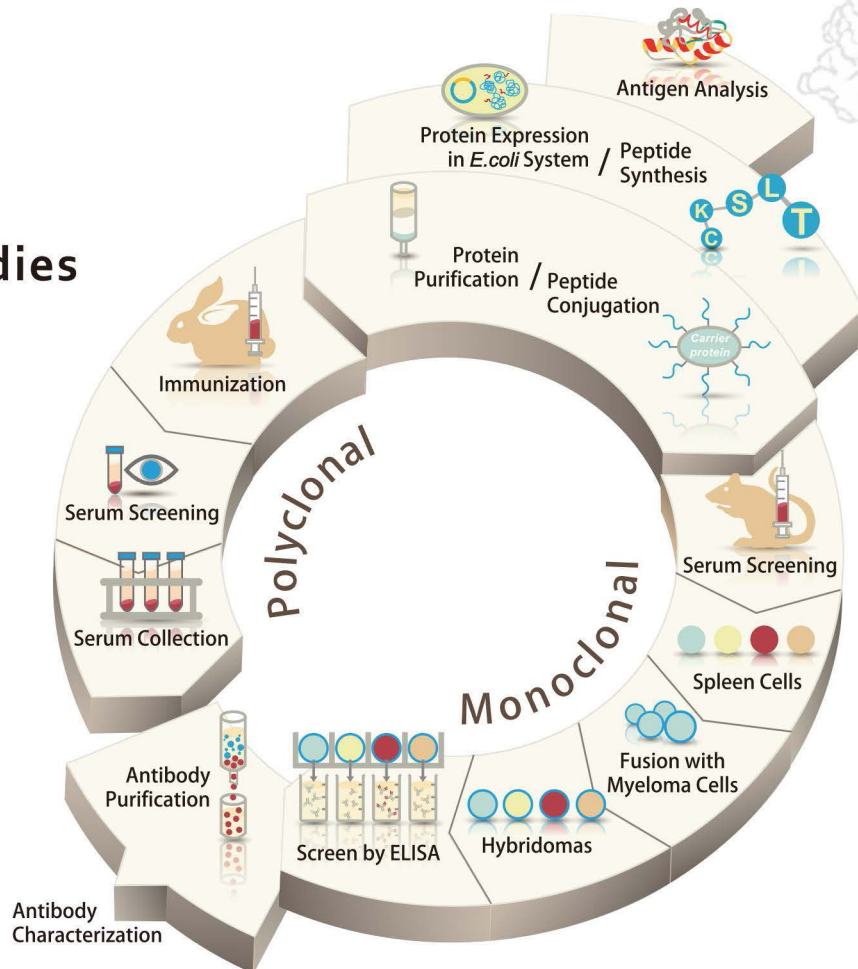
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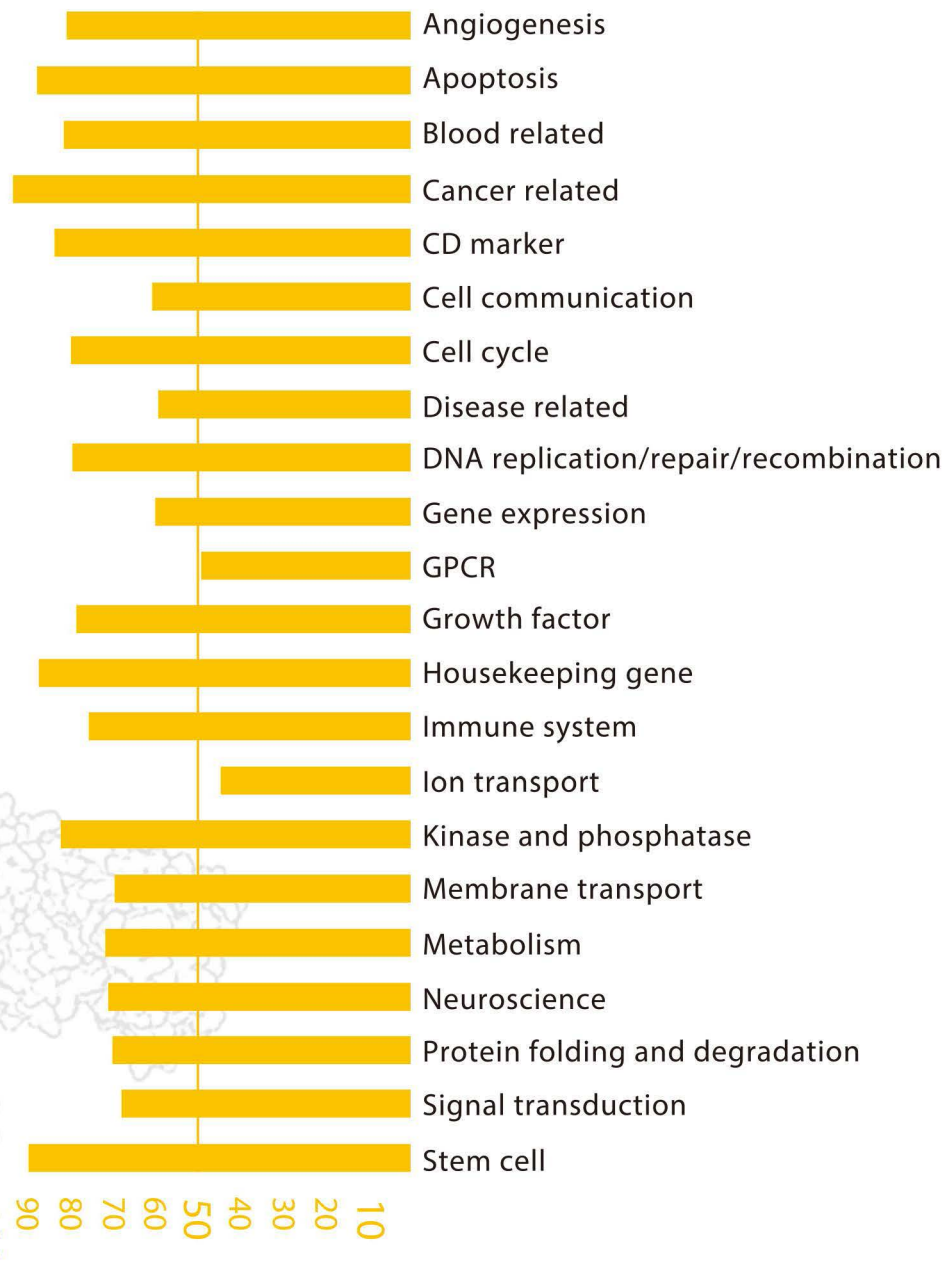
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