

ELISA

***(Enzyme - linked
Immunosorbent Assay)***

Steps for Sandwich ELISA

1. Coating with capture antibody
2. Incubation with blocking buffer
3. Addition of sample
4. Washing
5. Addition of primary antibody
6. Washing
7. Addition of secondary antibody
8. Washing
9. Addition of substrate
10. Measurement (if quantitative ELISA)

ELISA Formats

Capture (immobilization) of the antigen of interest:

- direct: adsorption to the assay plate
- indirect: via a capture antibody

Detection of the antigen:

- direct: labeled primary antibody
- indirect: labeled secondary antibody

Competitive Assay

- Used if antigen is small and has only one epitope
1. Labeling of purified antigen, coating of plate with capture antibody
 2. Unlabeled antigen from samples and labeled antigen compete for binding to primary antibody, which binds to capture antibody
 3. Decrease in signal from labeled antigen indicates presence of antigen in samples

Christian
Doppler
Laboratory

for
Cardiac and Thoracic
Diagnosis & Regeneration



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