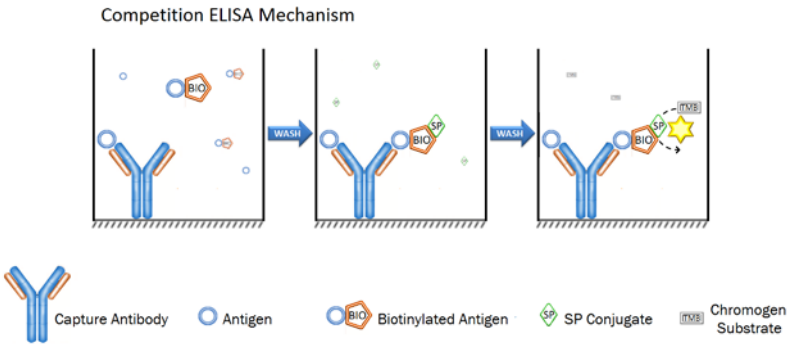
Introduction to ELISA
(Enzyme-Linked Immunosorbent Assay)

ELISA is a sensitive benchmark method for detection and quantification of an antigen in a sample. Competitive ELISA involves specific interaction between antigen and capture antibody that is highly purified, sensitive and specific. This relatively easy to use method shows accuracy and consistency with tested samples.

Competitive Enzyme Immunoassay

The antigen in standards and samples is competed with a biotinylated antigen sandwiched by the captured antibody and streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured. The intensity of color is directly proportional to the concentration of the antigen present in the sample.



Typical Competitive ELISA Kit Reagents

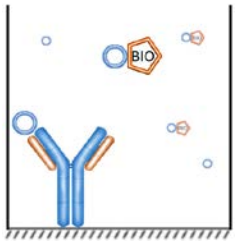
- Microplate coated with a capture antibody
- Standard (antigen of known concentration)
- Biotinylated Antigen
- Diluent
- Wash Buffer
- Streptavidin-Peroxidase Conjugate (SP conjugate)
- Chromogen Substrate (TMB)
- Stop Solution

Other Supplies Required

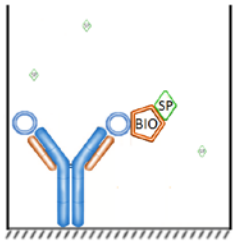
- Microplate reader capable of measuring absorbance
- Program with statistical calculator to perform linear regression analysis

Competitive ELISA Kit

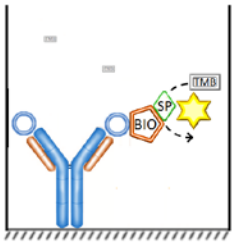
Step 1. Load standard or sample and biotinylated antigen onto microplate and incubate.



Step 2. Wash, then add SP conjugate and incubate.



Step 3. Wash, then add chromogen substrate and incubate till the optimal blue color density develops.



Step 4. Add stop solution and read at 450 nm.

General ELISA Procedure

- Load 25 µl of standard or sample per well and immediately add 25 µl of Biotinylated antigen to each well (on top of the standard or sample) and tap plate to mix gently. Incubate for 2 hours. Samples may need to be prediluted with diluent.
- Wash microplate five times with 200 µl of wash buffer.
- Add 50 µl of SP Conjugate per well and incubate for 30 minutes.
- Wash microplate five times with 200 µl of wash buffer.
- Add 50 µl of chromogen substrate per well and incubate till the optimal blue color density develops.
- Add 50 µl of stop solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.
- Use curve-fitting statistical program to plot a log-log or four-parameter logistic curve-fit to the standards and then calculate results for samples.

*** For *reference only; please follow the protocol provided with the ELISA kit for specific procedure suggested****

ELISA Kit Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are empty after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.

	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.
	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporated	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
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