ELISA Titer Determination

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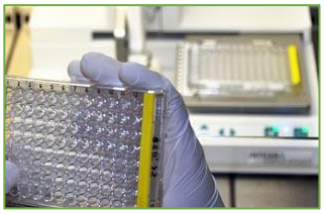
Introduction



To determine the concentration of specific antibodies in antisera the ELISA (Enzyme Linked Immuno-Sorbent Assay) titer determination is used. The antiserum is diluted in multiple steps. The last dilution step, which lead to a signal is the titer. The titer depends on the antigen used for the immunization and the immunization schedule. Not every antigen leads to a very good titer.

In the first step the specific antigen is coated to the ELISA plate. To block unspecific binding sites of the ELISA plate (where the first antibody could bind), proteins like BSA or milk powder are used to block these sites. The first antibody (i. e. antiserum or PrepI) binds the specific antigen. The more specific antibodies were produced, the more antibodies will bind. In the next step we add the second antibody that binds to the first antibody (for example anti-rabbit antibody). This second antibody is coated to an enzyme, like alkaline phosphatase. This enzyme can convert the colorless *p*NPP to the yellow PNP. The color can be measured in a photometer to determine the titer.

Material (for 3 plates)

Cat.No.	Name	Ingredients
D303 (50 ml)	Blocking Solution for ELISA	Davids Blocking Solution
D304 (100 ml)	ELISA Buffer	Davids ELISA Buffer
D302 (250 ml)	Incubation Solution for ELISA	Davids Incubation Solution for ELISA
	Secondary Antibody	Alkaline Phosphatase conjugated antibody Please dilute the secondary antibody in Incubation Solution
D305 (3 ml)	Substrate	10 x Solution of *p*NPP (p-nitrophenyl phosphate) D305 Please dilute the ELISA Substrate 1:10 in ELISA Buffer

Method

ELISA plate coating with antigen

Coat 100 ng antigen on each well on the plate. You may want to dilute the antigen prior to 0.1 M Bicarbonate pH 9. Incubate the plate for 24 hours at 4°C. Each antigen is different. Some may bind better some worse. Please try different coating concentration and conditions to receive the best results.

Washing Step

Wash three times with 200 µl 1 x PBS for 1 minute. Wash one time with 150 µl Incubation Solution D302 for 5 minutes.

Blocking

Add 150 µl Blocking Solution D303 in each well and incubate for 1 hour at 24°C.

First Antibody

Empty the ELISA plate and perform the “Washing Step” (see above). Add your first antibody. First lane with 1:10 dilution of your first antibody. Second lane with 1:50 dilution. Continue with a 5 times dilution to the end of the ELISA plate. Incubate the plate 24 hours at 4°C.

Secondary Antibody

Empty the ELISA plate and perform the “Washing Step”. Add 100 µl of the secondary antibody (i.e. Anti-Species-Antibody conjugated to alkaline phosphatase at a 1:2500 dilution) diluted in Incubation Solution and incubate for 2 hours at 24°C.

Washing

Empty the ELISA plate and perform the “Washing Step”. Before you perform the enzyme reaction, wash another time with ELISA Buffer D304 (150 µl each well) for 3 minutes.

Enzyme Reaction

Add 100 µl 1:10 diluted 10 x *p*NPP ELISA Substrate D305 and incubate the plate for 2 hours at 24°C. You can stop the reaction with 50 µl 2N NaOH. The plate can be read at 405 nm in an ELISA reader.

Trouble Shooting: No Signal

Antibody Concentration

Try to use different antibody concentrations. For raw sera you can use a dilution between 1:1.000 and 1:1.000.000. For antigen specific affinity purified antibodies, you may use an antibody concentration of 0.01 – 20 µg/ml. For ProteinA purified antibodies, you may use an antibody concentration of 0.01 – 50 µg/ml.

Incubation Time

The incubation time of the coating or the first or secondary antibody may be too short. Try to incubate them longer.

Secondary Antibody

Ensure that you use the correct secondary antibody. Anti-rabbit antibodies must be used when the antibodies were generated in rabbits for example. In addition, please have a look at the conjugated enzyme. HRP and alkaline phosphatase needs different substrates at the end.

ELISA Plate

The wells of the plate must never run dry. Please always keep liquid in the wells.

ELISA Reader

Please ensure that you use the correct wavelength, when you measure the reaction. For pNPP (p-Nitrophenyl Phosphate, Disodium Salt) you usually use 405 nm and for ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt)) 410 or 650 nm. OPD (o-phenylenediamine dihydrochloride) has an absorption maximum at 492 nm. Please ensure that no bubbles are in the wells when you measure the plate.

Substrate

Ensure you use the correct substrate for your secondary antibodies and always prepare the substrate fresh.

Detection Limit

Concentrate the samples or reduce the dilution in your ELISA.

Coating

You may need to conjugate your antigen to a carrier like BSA or Biotin to receive an optimal coating. You may even try different ELISA plates for optimal coating.

Buffer

Ensure that all buffers you use are compatible with your enzymes and antibodies. You may need different buffers for HRP and Alkaline Phosphatase Conjugated Antibodies.

Trouble Shooting: High Background

Washing

Please wash the wells at least three times according the protocol and prepare fresh wash buffer (PBS).

Blocking Buffer

Try another blocking buffer and keep the time according to the protocol. You may add small amounts of blocking buffer to the wash buffer to reduce the background.