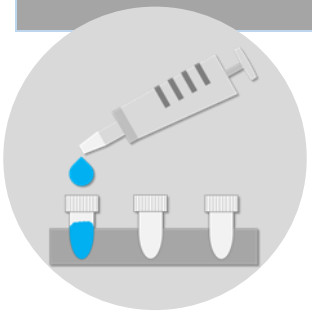
Davids Protocols



ELISA (Alkaline Phosphatase)

www.davids-bio.com (Custom Antibodies)
www.davids-science.de (Lab Material)

- 1 - Introduction

The ELISA (Enzyme-Linked Immunosorbent Assay) titer determination is utilized to measure the concentration of specific antibodies in antisera. The process involves diluting the antiserum in multiple steps, with the final dilution leading to a detectable signal representing the titer. The titer development is influenced by factors such as the antigen, the immunization schedule as well as the immunization route.

Initially, the ELISA plate is coated with the specific antigen. To prevent non-specific binding of the first antibody to the plate, blocking agents such as BSA or milk powder are utilized. The first antibody, which could be antiserum, affinity purified antibodies or PrepI, binds to the specific antigen. The number of antibody-antigen interactions that occur represents the amount of specific antibodies in the sample. In the subsequent step, a second antibody is introduced, which binds specifically to the first antibody (e.g., an anti-rabbit antibody). The secondary antibody is conjugated with an enzyme, in this protocol we use alkaline phosphatase. The enzyme can convert the colorless substrate *p*NPP into the yellow product *p*NP. The resulting color can be measured using a photometer to determine the titer of the antibodies.

- 2 - Material

Material		
Coating Buffer	0.1 M $NaHCO_3$ pH 8	Davids Coating Buffer Cat.No. 80.0010.30
Blocking Buffer	2% Milk Powder (BSA can be used as well) 0.5% Tween 20	Davids Blocking Solution Cat.No. 80.0012.50
Washing Buffer	1% NaCl 0.05% Tween 20	Davids Washing Buffer Cat.No. 80.0011.30
Dilution Buffer	1 x PBS 0.05% Tween 20	
Substrate	10 x Solution of *p*NPP (*p*-nitrophenyl phosphate) Please dilute the ELISA Substrate 1:10 in ELISA Buffer	
ELISA Plate	Use a high binding plate	Davids High Binding ELISA Plate

Secondary Antibody	Alkaline Phosphatase conjugated anti-species antibodies	www.davids-science.de

- 3 - Method

Coating

- Prepare 1 ml antigen solution for one row of the ELISA plate
- 1 µg antigen in 1 ml 0.1 M Davids Coating Buffer
- For small antigens like peptides, you may want to increase the concentration to 4 µg/ml
- Incubate the plate over night at 2 – 8°C

Washing (I)

- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

Blocking

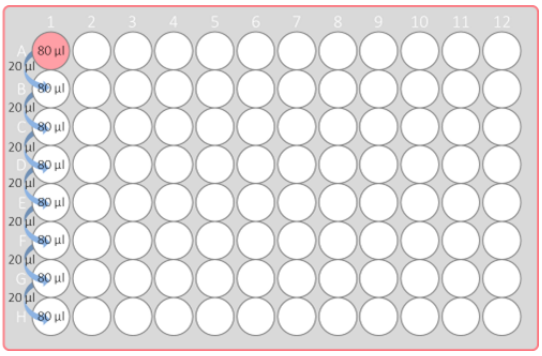
- Block ELISA plate with blocking buffer (i. e. 150 µl/well)
- Incubate the plate 30 min at room temperature

Washing (II)

- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

Primary Antibody

- For titer determination pipet 80 µl Dilution Buffer in each well
- For titer determination pipet 20 µl of your primary antibody (antisera or affinity purified antibodies produced by Davids with your antigen) in the first lane (red) and dilute the antiserum 1:5 by transferring 20 µl from the first lane to the second lane.
- Incubate the plate over night at 2 – 8°C



Washing (III)

- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

Secondary Antibody

- Dilute the secondary antibody in Dilution Buffer
- Dilute the secondary antibody according to the manual
- Pipet 100 µl of the diluted antibody solution in each well
- Incubate the secondary antibody for 2 hours at room temperature

Washing (IV)

- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

Substrate (HRP)

- Pipet 100 µl 1 x substrate solution (*p*NPP) in each well
- Incubate plate for 2 hours on the shaker at room temperature
- Read the plate at 405 nm

- 4 - Trouble Shooting

Reason	Solution
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.
Primary 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.
Detection Limit	Concentrate the samples or reduce the dilution in your ELISA.
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 use 405 nm. Please ensure that no bubbles are in the wells when you measure the plate.
Incubation Time	The incubation time of the coating or the first or secondary antibody may be too short. Try to incubate them longer.
Blocking Problems	You may want to block with BSA instead of milk powder to get a better response. You may add small amounts of blocking buffer to the wash buffer to reduce the background. Try to add or change the concentration of Tween 20 to your blocking solution for better results.
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.
Substrate	Ensure you use the correct substrate for your secondary antibodies and always prepare the substrate fresh.

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