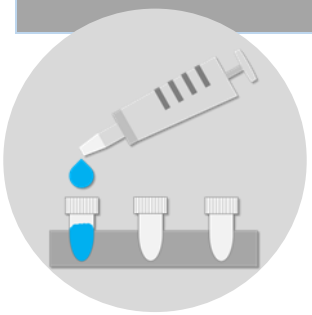
Davids Protocols



ELISA (HRP)

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

- 1 - Introduction

ELISA (Enzyme-Linked Immunosorbent Assay) is a technique that enables the detection and quantification of antibodies, antigens, or other proteins within a sample. Its versatility extends across various applications, including medical diagnostics for identifying infections such as HIV or hepatitis. The food industry makes use of ELISA to detect food allergens, while environmental monitoring relies on it to identify pollutants like pesticides and heavy metals. Furthermore, ELISA serves as a standard research method for quantifying specific proteins in biological samples and studying the immune system.

In this protocol, the ELISA technique is utilized to determine the titer, which is an indirect method of measuring the concentration of specific antibodies in a sample, which could be the antiserum or PrepI from egg yolks. The titer refers to the highest dilution of an antiserum that still produces a detectable response in the ELISA assay.

- 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	1 mg TMB in 0.1 ml DMSO Add 9.9 ml Na-Acetate pH 6 Add 0.01% H_2O_2 Substrate must not precipitate	
ELISA Plate	Use a high binding plate	Davids High Binding ELISA Plate

- 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

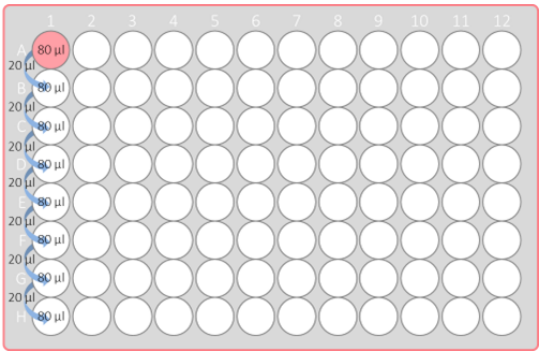
- Block ELISA plate with blocking buffer (i. e. 150 µl/well)
- Incubate the plate 30 min at room temperature

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 for 1 hour at room temperature on the shaker



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 for 1 – 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 substrate solution (TMB) in each well
- Incubate plate for 15 - 30 minutes on the shaker
- Add 100 µl Stop Solution (0.1 M H2SO4)
- Read the plate at 450 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 TMB use 450 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).