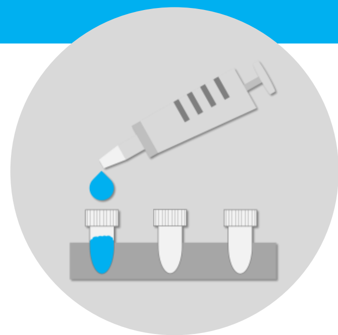
DAVIDS PROTOCOLS

Davids Biotechnologie



ELISA

www.davids-bio.com

Material

Blocking Buffer	Washing Buffer	Coating Buffer	Dilution Buffer
Davids Blocking Solution Cat.No. 80.0012.50 Alternative: • 2% Milk Powder or BSA • 0.5% Tween 20	Davids Washing Buffer Cat.No. 80.0011.30 Alternative: • 1% NaCl • 0.05% Tween 20	Davids Coating Buffer Cat.No. 80.0010.30 Alternative: • 0.1 M $NaHCO_3$ pH 8	1 x PBS 0.05% Tween 20

Substrate (HRP)	Secondary Antibody	ELISA Plate
• 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	www.davids-science.com: For rabbit antibodies For chicken antibodies For mouse antibodies	Davids High Binding Plate

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 at 2 – 8°C over night

Washing Step I

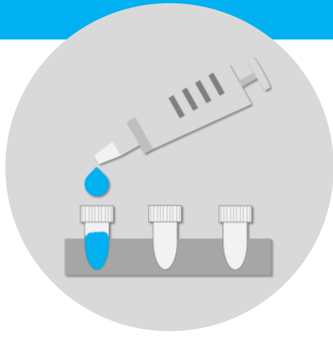
- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

Blocking

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

Washing Step II

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



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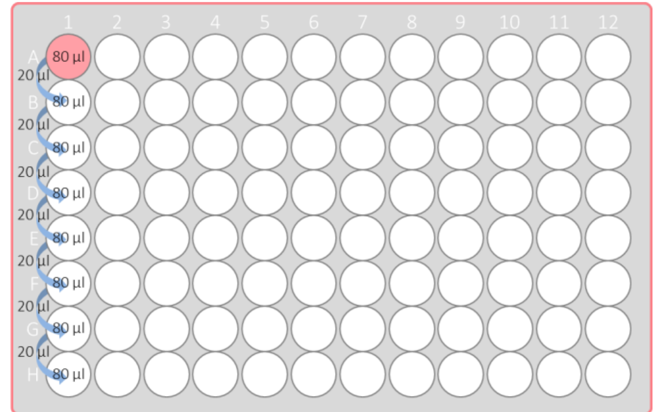
Primary Antibody

- 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 Step III

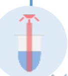
- Wash ELISA plate with washing buffer (i. e. 200 µl/well)
- Incubate for 5 minutes at room temperature
- Repeat Washing step 3 times

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

Washing Step 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 H_2SO_4)
- Read the plate at 450 nm

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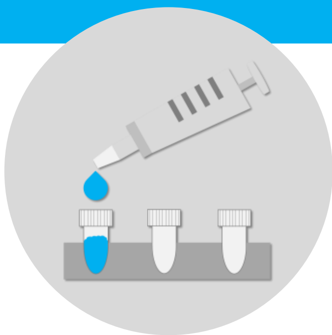

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No Signal

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.
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.
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.

DAVIDS TROUBLESHOOTING

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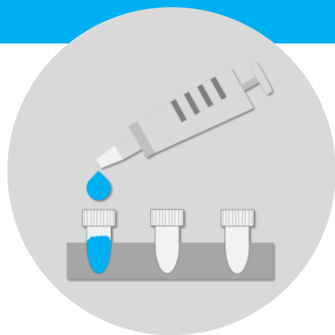

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High Background

Reason	Solution
Analyte Concentration	
Antibody Concentration	
Incubation of primary antibody	
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.
Blocking Problems	Try to add or change the concentration of Tween 20 to your blocking solution for better results.
Washing	Please wash the wells at least three times according the protocol and prepare fresh wash buffer (PBS).
Substrate	
Membrane Handling	