Protocol

Peptide ELISA

Ready-to-use peptide ELISA

Revision 1.2

Contact us:

Support:	+49-30-6392-7878
Order per fax:	+49-30-6392-7888
Or e-mail:	peptide@jpt.com
www:	**www.jpt.com**

JPT Peptide Technologies GmbH
Volmerstrasse 5
12489 Berlin
GERMANY

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1 Introduction

Humoral immune response can be displayed by circulating antibodies that recognize a vast repertoire of specific antigens. The epitope pattern of relevant antigens recognized by antibodies is patient-specific and may be altered by disease progression, therapeutic intervention and other circumstances. The so called “epitope spreading” is observed in cancer, infectious and autoimmune diseases and allergies and is an indication for changes and activation of the immune repertoire. Epitope discovery, deconvolution of epitope changes and epitope spreading may lead to new biomarkers, tools for individualized therapeutic vaccine strategies, patient stratification and enable the correlation of humoral immune response with clinical outcome.

Peptide ELISA is an efficient tool for mapping the immune response at a single epitope resolution. For the assay, the plate is incubated with a biological sample (e.g. blood serum/plasma or saliva). Circulating antibodies that bind specifically to the peptides are detected by a labeled secondary antibody. The use of peptide ELISA is not limited to the detection of antibody signatures directed against foreign antigens in infectious diseases and allergies. It can also be used to detect antibodies directed to tumor associated antigens, mutations and post translational modifications. The correlation of humoral immune responses with clinical effect is an important issue since it represents the gateway to novel diagnostics and therapies.

Protein based assays measure binding events of circulating antibodies at the protein level. In contrast, peptide ELISA provides high resolution information about the antibody repertoire down to distinct epitopes. Thus, this approach allows monitoring of individual epitope patterns as well as epitope spreading and addresses natural sequence diversity and post translational modifications.

Further, peptide ELISA can be used for investigation of protein-protein interactions and examination of the enzymatic activities (e.g. of enzymes mediating posttranslational modifications).

2 List of components

Component	Quantity
Peptide ELISA	One 96 well plate One lid

Biotinylated peptides were immobilized on streptavidin coated polystyrene microtiter plates. The peptides were separated from the biotin moiety via a hydrophilic linker.

JPT's *Peptide ELISA plates* were designed with focus on applicability for a variety of different sample types and compatibility with many distinct assay protocols. In order to prevent interference of the blocking agent with customer's samples and assay setup, the *Peptide ELISA plates* were not blocked. For instance, anti-BSA antibodies can be found in some human serum and plasma samples. Therefore, using BSA for blocking would result in a high background for these samples. For the most applications, JPT is using SuperBlock T20 (TBS) Blocking Buffer, Thermo Scientific, #37536 for blocking and secondary antibodies. For the dilution of serum samples Low Cross Buffer (LowCross Buffer, Candor Bioscience, order # 100050) is recommended.

3 Storage and handling

3.1 Storage of Peptide ELISA plates

- Optimal storage conditions for peptide ELISA plates are in a cool (approx. 4 °C / 39 °F), dark and dry environment.
- Peptide ELISA plates are stable for at least six months when stored at 4 °C (39 °F).
- Do not freeze the peptide ELISA plates.

3.2 Handling of Peptide ELISA plates

- Always handle the *Peptide ELISA plates* with care.
- Always wear laboratory gloves when handling *Peptide ELISA plates*.
- Hold *Peptide ELISA plates* at the sides. Keep the down side of the plate clean. Avoid scratches.
- Take care when dispensing solutions into the wells. Make sure not to touch the surface with pipette-tips or dispensers.
- Inappropriate chemicals may destroy the bonding of the peptides to the surface. Never use chemicals with corrosive activity.
- Preferably filter all solutions for the washing steps through 0.2 µm particle filter before use.

READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS! CAREFULLY NOTE THE HANDLING AND STORAGE CONDITIONS OF JPT's PEPTIDE ELISA.
PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES´ CUSTOMER SUPPORT FOR ASSISTANCE IF NECESSARY.

4 Experimental protocols

Note: The following procedure is given as a guideline only. The optimal experimental conditions will vary depending on the investigated sample and instruments used and can, therefore, not be predetermined. The optimal experimental conditions must be established by the user. No warranty or guarantee of performance using this procedure with your target antibody or serum can be made or is implied.

4.1 Additional materials and solutions required

Component	Recommendations / Remarks
Primary antibody:	Final concentration of about 1-10 µg/ml.
Proteins / enzymes:	For analysis of e.g. binding or modifying components, JPT recommends a final concentration of 10 µg/ml or above.
Blood sera or plasma solution:	Final sample dilution of 1:100 to 1:1000 in blocking reagent or assay buffer.
Secondary antibody:	JPT recommends a peroxidase labeled secondary antibody at a final conc. of about 0.2-2 µg/ml. Depending on your experimental setup, alkaline phosphatase and fluorescently labeled secondary antibody can also be used.
Wash buffer:	JPT recommends 1x TBS-Buffer + 0.05% Tween 20 (TBS-T). Depending on the application, also a PBS based washing buffer can be used.
Blocking buffer	Depending on the application, a Casein or BSA based blocking buffer can be used.
Detection:	When using a peroxidase labeled secondary antibody, JPT recommends TMB (3,3',5,5'-Tetramethylbenzidine) as substrate and 25% sulfuric acid as stop solution. Depending on your equipment and detection label, a variety of different substrates and detection techniques can be applied.

4.2 Additional hardware

Component	Recommendations / Remarks
Microplate washer	For efficient and repeatable washing, JPT recommends the usage of a microplate washer.
Microplate reader	Depending on the used detection system, an appropriate microplate reader is required.
Microplate shaker	JPT recommends the usage of a temperature controlled microplate shaker.

4.3 General principles for incubation

I. BLOCKING	Apply 300 µl (per well) blocking buffer Incubate for 1 hour at 30 °C in a shaking incubator at 300 rpm
II. EMPTY	Remove the blocking buffer
III. INCUBATION WITH PRIMARY ANTIBODY / ANALYTE	Apply 100 µl (per well) of your analyte diluted in blocking buffer Incubate for 1 hour at 30 °C in a shaking incubator at 300 rpm
IV. WASHING	Wash 4x 300 µl (per well) TBS-T
V. INCUBATION WITH SECONDARY ANTIBODY	Apply 100 µl (per well) of your secondary antibody diluted in blocking buffer Incubate for 1 hour at 30 °C in a shaking incubator at 300 rpm
V. WASHING	Wash 4x 300 µl (per well) TBS-T
VI. DETECTION	This step depends on the secondary antibody label and equipment available. JPT recommends usage of a peroxidase labeled secondary antibody with TMB substrate and an absorption microplate reader.

Exemplary detection protocol:

- Apply 100 µl TMB solution (per well)
- Incubate for 6 minutes at 30 °C. Time course of the color reaction can be recorded at 655 nm (absorption).
- Apply 100 µl 25% sulfuric (per well) as stop solution. Measure absorption at 450 nm.

The optimal incubation time will be in a range of 3-30 min and must be determined empirically.

5 Notes / Troubleshooting

Comments and Suggestions

High background	• Carefully adjust the final dilution of your analyte. • Carefully adjust the final dilution of your labeled secondary antibody. • Try a different blocking buffer like the LowCross Buffer from Candor Bioscience (order # 100 500). • Ensure efficient washing. • Detection step, reduce the incubation time for the substrate. Add stopping solution at an earlier time point.
Reproducibility	• For the incubation with the first and second antibody, tightly control all incubation parameters like time and temperature. • For the detection of enzymatic activity, tightly control the timing and temperature before stopping the reaction.
Unspecific signals	• Control incubations using labeled secondary antibody alone should be performed in parallel to the actual experiment to ensure that found signals are not caused by non-specific binding of the secondary antibody to the immobilized peptides.
Little or no signals	• Ensure sufficient incubation time. • When using a peroxidase-based readout, make sure your buffers do not contain azide. • When using alkaline phosphatase, do not use PBS based buffers.

6 Related products

For further information visit our homepage (www.jpt.com) or contact our customer support.

- Histone Code Peptide ELISA
- PepStar™ High Content Peptide Microarray
- PepStar™ Multiwell Peptide Microarray
- RepliTope™ Catalog Microarrays
- BioTides™ Biotinylated Peptides