Protocol

BioTides™ Peptides

ELISA plate coating with synthetic peptides prepared by SPOT-Synthesis

Revision 1.0

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Table of content

1 Introduction and Experimental Basics

BioTides™ biotinylated peptides are designed for biomedical assays that require immobilization onto streptavidin coated beads, membranes, glass slides or microtiter plates (e.g. Peptide ELISA). BioTides™ are synthesized by JPT's proprietary SPOT synthesis technology providing fast access to large numbers of biotinylated peptides at unmatched pricing.

Properties of BioTides™ Biotinylated Peptides:

- Each peptide is biotinylated at the N-terminus
- A flexible hydrophilic linker between biotin and peptide is inserted
- The specialized synthesis method results in automatic removal of truncation sequences during immobilization
- Incorporation of non-natural amino acids or post-translational modifications like methylation or phosphorylation possible

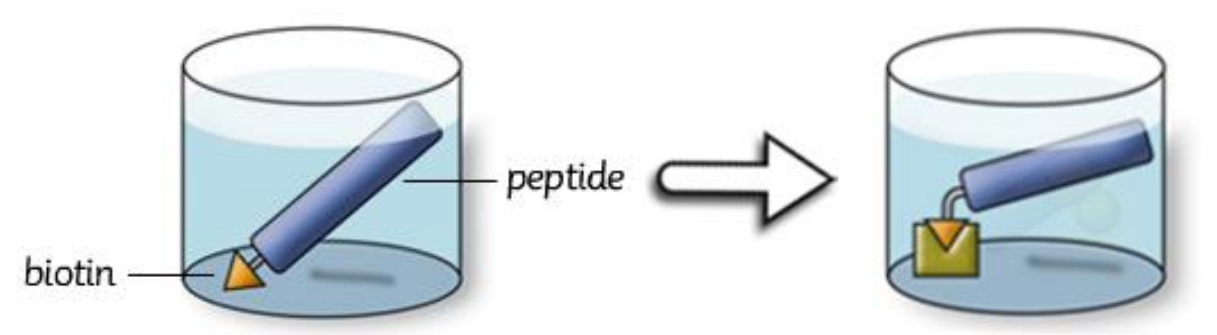

Fig.1: N-terminally biotinylated Peptide immobilized onto micro titer plates.

BioTides™, immobilized on streptavidin coated microtiter plates can be used to perform Peptide ELISA (Enzyme-linked immunosorbent assay), an efficient tool for mapping the immune response at a single epitope resolution.

2 List of Components

Component	Quantity
BioTides™ Biotinylated Peptides	Quantity according to order
Datasheet including plate layout and sequence information	One datasheet per batch
Leaflet including relevant information for storage and handling	One leaflet per batch

3 Storage and Handling

- Optimal storage conditions for undissolved peptides are in a cool (approx. 4°C / 39°F), dark and dry environment.
- Dissolved peptides should be stored at -80°C but might be subject to degradation.

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING YOUR EXPERIMENTS!
PLEASE CONTACT JPT PEPTIDE TECHNOLOGIES´ TECHNICAL SERVICES FOR ASSISTANCE IF NECESSARY.

4 Additional Materials Required

4.1 Additional Reagents

- Dimethylsulfoxid (DMSO)
- 10x Phosphat buffered saline (800g NaCl, 20g KCL, 144 g Na2HPO4 · 2H2O, 24 g KH_2PO_4 8 L of distilled water)
- Peptide coating buffer: JPT recommends filtered 1x Phosphat buffered saline with 40 % DMSO and 0.05 % Tween)
- Wash-buffer: JPT recommends filtered 1x Phosphat buffered saline with 0.05 % Tween 20 (JPT commonly used Tween 20 from Sigma Aldrich - P7949)
- Blocking-buffer: JPT recommends filtered 1x Phosphat buffered saline with 400 µM Biotin and 20 % Sucrose (JPT commonly used Biotin from AppliChem – A0969 and Sucrose from Sigma Aldrich - 84097)

4.2 Additional Hardware

- Streptavidin coated Microtiter Plates: JPT recommends steptavidin coated plates from Thermo Fisher – 436014
- 96-Well Deep Well Plate
- Microplate shaker: JPT recommends the usage of a temperature controlled microplate shaker.
- Microplate washer: For efficient and repeatable washing, JPT recommends the usage of a microplate washer.

5 Coating Protocol

Note: The following procedure is given as a guideline only. The optimal experimental conditions will vary depending on the streptavidin coated microtiter plates and instruments used. Therefore, it cannot be predetermined. The optimal experimental conditions must be established by the user. No warranty or guarantee of performance using this procedure can be made or is implied.

The following coating procedure has been developed exclusively for streptavidin-coated microtiter plates.

5.1 Dissolve Biotinylated Peptides

Remove the protective silicon seal from the delivered plate and add 100 µl DMSO to each peptide containing well. The plate layout and sequence information are delivered together with this protocol. Shake the plate until each peptide is fully dissolved, resulting in peptide stock-solutions (1).

5.2 Dilution

Prepare a 250 fold dilution of each peptide stock-solution in a separate 96-Well Deepwell plate (1) using peptide coating buffer (see section 4.1), resulting in peptide-solution (2).

5.3 Prewash

Depending on the streptavidin coated plates used, it is recommended to perform a prewash step of the full microtiter plate with wash-buffer (see section 4.1). Further information should be provided by the microtiter plate supplier.

5.4 Immobilization

Add 100 µl of each peptide-solution (2) to a streptavidin-coated microtiter plate well according to your preferred assay-plate layout. Incubate the plate for 1 hour at room temperature without shaking.

5.5 Washing

Wash the plate 4 times with 300 µl wash-buffer per well and empty the plate after the last wash.

5.6 Blocking

Add 200 µl blocking buffer (see section 4.1) to each peptide coated well and incubate the plate for 30 min at room temperature without shaking. Afterwards, remove the blocking solution, but do not wash the wells.

5.7 Drying and packaging

Dry the microtiter plates upside down on a paper towel at room temperature. If the plates are not used immediately it is recommended to store them at 4°C after packaging in aluminium bags together with a dry sac.

6 Related Products

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

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