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ELISA Method Using Biotinylated Peptides

Introduction

The use of peptides in solid-phase immunoassays requires an efficient method for immobilization of the peptides on the solid phase, i.e. a method which does not depend on the amino acid sequence of the peptide being tested [1]. For this purpose, Mimotopes has developed the use of biotinylation of multiple synthetic peptides, and subsequent attachment of the peptides to a plastic surface coated with Avidin, NeutrAvidin™ or streptavidin [2]. This article deals with the practical details of carrying out a successful ELISA with such biotinylated peptides.

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Materials Required

Mimotopes biotinylated synthetic peptides

Streptavidin coated plates, pre-blocked with BSA (Pierce Cat. No. 15125)

Phosphate Buffered Saline (PBS, 0.01M)

Bovine serum albumin (BSA, Pierce Cat. No. 37525)

Sodium azide PBS/Tween 20 (0.1% Tween 20 in PBS)

PBS/BSA/azide (0.1% BSA and 0.1% sodium azide in PBS)

2% BSA/PBS (PBS containing 2%BSA) Conjugate Substrate

ELISA Plate reader

Method

1. The biotinylated peptides are supplied as a dry powder in Micronic tubes (Cat. No. M42001). The identity of the peptide in each tube is given in the information supplied with the peptides. For use, we recommend that the peptides are reconstituted in 200 microlitre of either a pure solvent (e.g. dimethyl sulfoxide or dimethyl formamide) or solvent/water mixture. Each peptide should be diluted just before use to a working strength of 1/1000 in PBS/BSA/azide, i.e. PBS containing 0.1% BSA and 0.1% sodium azide. The peptide stock solution may be diluted further (down to 1/5000), however some loss in ELISA sensitivity may occur if used too dilute.
2. An initial 1/100 dilution is conveniently made using 1mL capacity polypropylene tubes, held in an 8x12 format rack (Bio-Rad Cat. No. 223-9390 as used for supply of the peptides is suitable). Using a multichannel pipette, transfer a 10uL aliquot of peptide solution into each tube, then add 1mL of the PBS/BSA/azide, cap the tubes and invert several times to ensure thorough mixing. The diluted peptide solutions may be stored for several days at 4 degrees C. For longer storage the diluted peptide solutions should be stored frozen. The tubes containing the supplied peptides should be resealed and stored frozen immediately after sampling, preferably at -70 degrees C.
3. To react the streptavidin coated, BSA blocked plates with the biotinylated peptides, transfer 100 microlitre of each of the diluted peptide solutions into the corresponding well positions of the plate, place the plate on a shaker and allow the reaction to proceed for 1hr at 20 degrees C. For convenience, several sets of immobilised peptides may be prepared simultaneously.
4. Wash plates with Phosphate Buffered Saline (PBS, 0.01M sodium phosphate in 0.15M sodium chloride, pH7.2) containing 0.1%(v/v) Tween 20 (PBS/Tween 20). The washing technique is as follows: flood the plate, filling all the wells with solution, then vigorously flick the solution from the wells. This washing step is repeated 4 times. After the washings, excess buffer is removed from the wells by vigorously “slapping” the plates, wells down, on a benchtop covered with an absorbent material (paper towels).

   The plates should be dried at 37 degrees C before storage at 4 degrees C in the dry state if they are not to be used immediately.
5. Dilute the serum to be tested, using as diluent 2%BSA/PBS containing 0.1% sodium azide. The optimum dilution of the serum will depend to some extent on the source and the level of antibodies present in the sample. The recommended dilutions are 1/1000 for hyperimmune serum from experimental animals and ascites fluid from hybridoma-bearing mice, and 1/500 for human serum. Add 100 microlitre of the diluted serum to each of the wells of the plate containing

captured peptides. Place the plate on a shaker and incubate for 1hr at 20 degrees C or overnight at 4 degrees C. Better sensitivity has been observed for some antibodies with the overnight reaction.

6. Repeat washes as described in Step 4.
7. Bound antibody is detected after reaction for 1hr at 20 degrees C with 100 microlitre conjugate solution, comprising a saturating level of horseradish peroxidase-labeled anti-species antibody (we have found a 1/2000 dilution of the 1mg/mL stocks from Pierce, to be suitable) made up in 2%BSA/PBS. Note: do not use a diluent containing azide for HRP conjugates.
8. Repeat washes as described in Step 4.
9. Wash the plate twice with PBS only, to remove traces of Tween remaining from the washing buffer.
10. The presence of enzyme is detected by reaction for up to 45min at 20 degrees C with 100 microlitre/well of 1-Step™ ABTS (Pierce Cat. No. 37615). Using this substrate the absorbance of the converted substrate solutions (product) in each well is read using a microtitre plate reader. The Titertek Multiskan MC plate reader in the dual wavelength mode at 405nm against a reference wavelength of 492nm is suitable. The absorbance values are recorded and stored on a diskette for later analysis.

Notes

NeutrAvidin™ coated plates (Pierce Cat. No. 31000) are also available from Pierce and may provide lower background levels.

Other ELISA enzyme/substrate systems may be used, but the sensitivity of the test will vary accordingly. The conditions for each ELISA system should be optimised (substrate concentration, pH, temperature, time etc.).

A test should be performed using preimmune, negative or normal serum to verify that any binding observed was due to specific antibodies. Likewise, a negative control test can be performed with direct addition of conjugate to plates coated with (strept)avidin-peptide to ensure that the positives are not due to conjugate binding directly to the peptide.

If you have any queries or difficulties, please contact Peptide_Technical_Support@mimotopes.com.

References

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2. Weiner, A. J., Geysen, H. M., et al. (1992) Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: Potential role in chronic HCV infections. Proc. Natl. Acad. Sci. USA 89; 3468-3472.

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