## **Peptides** and Immunology

**User Note** 

## **TEST PROCEDURE FOR CONTROL** ANTIBODY AND POSITIVE AND **NEGATIVE TEST PEPTIDES**

The aim of this test is for the operator to become familiar with the ELISA using biotinylated peptides prior to carrying out assays on his or her set of biotinylated peptides. See the attached data sheet for the control monoclonal antibody details.

NOTE: The amount of control peptides supplied is less than that present for all peptides as they have been prediluted for convenience. For all other peptides follow the instructions presented in the general assay procedure.

## **Test Protocol**

1. Dissolve the control positive and negative peptides e.g. using 1mL of 40% Acetonitrile/Water solution, or if acetonitrile is unavailable, using pure water.

2. Transfer the total contents of the tube to a small bottle and add 9mL of PBS/Tween®20/azide solution (see enclosed notes for details of this solution) and mix well.

3. These biotinylated peptide solutions are then used without further dilution for capture onto the coated streptavidin or avidin plates. The peptide capture conditions are as described in the general assay procedure. Using a well volume of 100µL there is enough peptide solution to saturate one avidin or streptavidin coated microtitre plate. The operator however may wish to only add peptide to a few wells. Unused peptide solution should be stored at -20°C.

**4.** After the peptide capture, follow the general assay procedure presented in the accompanying documentation, using the reconstituted control antibody supplied and an anti-mouse immunoglobulin conjugate.



p1

PU3-003-2

The attached figure is a typical titration test result obtained where the control antibody was tested at a number of dilutions on the positive and negative control peptides.

NOTE: The absolute ELISA values obtained may vary from those shown and will be dependent on many factors, such as time of incubation, detecting conjugate and substrate. However, the ELISA readings for the positive and negative control peptides should be clearly differentiated as shown

## TYPICAL ELISA PLOT USING CONTROL ANTIBODY



11 Duerdin St, Clayton VIC, Australia 3168 mimotopes@mimotopes.com australia@mimotopes.com www.mimotopes.com

Mimotopes International Mimotopes Asia Pacific Tel: +61 3 9565 1111 Fax: +61 3 9565 1199

**Mimotopes Europe** Tel: +44 870 460 1500 Fax: +44 870 460 1501 europe@mimotopes.com Mimotopes US East Tel: +1800 633 8161 Fax: +1800 424 3970 useast@mimotopes.com **Mimotopes US West** Tel: +1800 644 1866 Fax: +1800 655 1866 uswest@mimotopes.com

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