ELISA titre determination - general protocol

Wells are coated with the immunizing peptide and serially diluted antibodies are applied and measured.

- Coat 0.1ug peptide in 100ul PBS per well on high binding ELISA plates overnight at 4C.
- Wash 3 times with 300ul PBS per well.
- Block with PBS/0.1%(v/v) Tween-20 (PBST) containing 3%(w/v) skimmed milk (blocking buffer) for 1h at 37C
- Wash 3 times with PBST
- Make serially dilutions of antibody in blocking buffer and apply 100ul per well.
- Incubate 1h at 37C with the plates covered
- Wash three times with 300ul PBST
- Apply secondary antibody (AP-conjugate) at appropriate dilution in blocking buffer, 100ul per well
- Wash four times in 300ul PBST
- Wash 2 times in 300ul PBS
- Add 50ul per well p-nitro phenyl phosphate (pNPP) from Sigma and incubate for 30min at 30C
- Stop the reaction with 50ul 1M NaOH.
- Read at 405nm

Phosphate buffered saline (PBS): 20mM Sodium phosphate, pH7.4 in 150mM NaCl.