

Antibody Pairs Protocol

The matched pair antibodies have been tested using the following protocol.

1. Buffers and Reagents

Carbonate Coating Buffer (100 mM)

Antibody should be diluted in coating buffer to immobilize them to the wells:

3.03 g Na₂CO₃

6.0 g NaHCO₃

Make up to 1000 ml with distilled water and adjust to pH 9.6.

PBS

1.16 g Na₂HPO₄

0.1 g KCl

0.1 g K₃PO₄

4.0 g NaCl

Make up to 500 ml with distilled water and adjust to pH 7.4.

Wash Solution - PBST (0.1% (v/v) Tween)

Add 5 ml 10% (v/v) Tween-20 stock solution to 500 ml PBS.

Blocking and Antibody/Protein Dilution Buffer

5% (w/v) BSA in PBST.

Stop Solution

0.25M Sulphuric Acid.

2. Protocol

1. 96-well ELISA plates are coated with 100µl/well of **Capture Antibody** diluted to the recommended concentration in 100mM **Carbonate Coating** buffer, pH9.6.
2. Incubate the plates overnight at 4°C for 1 hour at room temperature to allow adsorption of the Capture Antibody to the plate.

3. Remove the **Capture Antibody** and wash plates 3 times with **PBST** (3x300µl/well), leaving the plates to dry after the final wash.
4. Block plates with 150µl/well of **Blocking Buffer**.
5. Incubate plates at room temperature for 1 hour.
6. Aspirate **Blocking Buffer** and add 50µl/well of **Target Protein** at the recommended concentration in **Blocking Buffer**.
7. Incubate for 1 hour at room temperature.
8. Remove the **Protein Solution** and wash plates 3 times with **PBST** (300µl/well), leaving the plates to dry after the final wash.
9. Add 50µl/well of **Detector Antibody** diluted to the recommended concentration in **Blocking Buffer**.
10. Incubate for 1 hour at room temperature.
11. Remove the **Detector Antibody** solution and wash plates 3 times with **PBST** (300µl/well), leaving the plates to dry after the final wash.
12. Aliquot 100µl/well of anti-rabbit HRP conjugate **Secondary Antibody** diluted 1:10,000 in **Blocking Buffer**.
13. Incubate for 1 hour at room temperature.
14. Remove the Detector Antibody solution and wash the plates 3 times with **PBST** (300µl/well), followed by 2 washes with **PBS**, leaving the plates to dry after the final wash.
15. Add 50µl/well of **TMB Peroxidase Substrate**.
16. Incubate for 10 minutes at room temperature (The length of this incubation may be optimised, but 10 minutes works well in most cases).
17. Add 50µl/well of 0.25M Sulphuric Acid **Stop Solution**.
18. Read plates on plate reader at 450nm.