Matched Antibody Pair Set Protocol

Detailed sandwich ELISA workflow for antibody pair sets

Purpose: This protocol contains the process details intentionally kept out of the IFU: reagent preparation, plate coating, blocking, standard preparation, sample incubation, detection, signal development, and calculations.

Research-use limitation: For research use only. Not intended for diagnostic use.

1. Safety and Handling

- Some components may contain ProClin®, which may cause allergic skin reaction or respiratory irritation.
- Stop Solution is a concentrated acid solution. Use appropriate PPE.
- TMB substrate may cause skin, eye, and respiratory irritation. Wear PPE and wash hands after handling.
- Review the relevant Safety Data Sheet before use.
- Capture antibody may contain sodium azide. Do not dispose leftover material directly into sinks.

2. Required Equipment

- Microplate reader capable of measuring absorbance at 450 nm.
- Multi-channel and single-channel pipettes.
- Recommended: plate shaker or rocker.

3. Materials Required but Not Supplied

- Nunc™ MaxiSorp™ 96-well plates or equivalent high-bind 96-well microplates.
- Plate seals.
- Coating Buffer: 35 mM $NaHCO_3$, 15 mM Na_2CO_3, pH 9.6.
- Blocking Buffer: dilute 20X stock in PBS to 1X; 1% BSA, 0.05% Tween® 20 in 1X PBS, pH 7.2-7.4. BSA purity of ≥96% is recommended.
- Wash Buffer: dilute 20X stock in water to 1X; 0.05% Tween® 20 in 1X PBS.
- TMB substrate and Stop Solution.
- 20X PBS diluted to 1X as needed.
- Streptavidin-HRP Solution for biotin-labelled detector antibody workflows.
- Microcentrifuge tubes for standard dilution and double-distilled water.

4. Reagent Preparation

- Equilibrate all reagents to room temperature, 18-25°C, before use.

- After initial reconstitution, allow reagents to sit for at least 15 minutes with gentle shaking. Mix well before use.

- Prepare reagent dilutions immediately before use and prepare only the amount needed for the experiment.

Reagent	Preparation	Volume
Capture antibody	Dilute stock capture antibody to 0.5-2 µg/mL in Coating Buffer. Optimize as needed.	100 µL/well
Detector antibody	Dilute biotin-labelled detector antibody to 0.5-2 µg/mL in Blocking Buffer or validated diluent. Optimize as needed.	100 µL/well
Streptavidin-HRP	For biotin detector workflows, dilute to 0.05-0.2 µg/mL in 1X Blocking Buffer. Omit this step for HRP-conjugated detector antibody workflows.	100 µL/well

5. Plate Preparation

1. Add 100 µL of capture antibody to each well of a high-bind 96-well microplate.

2. Seal the plate and incubate overnight at 4°C or for 2 hours at 37°C.

3. Wash the plate three times with 300 µL of 1X Wash Buffer. Remove liquid completely after the last wash by tapping the plate against absorbent towels.

4. Add 300 µL of 1X Blocking Buffer to each well. Seal and incubate overnight at 4°C or for 2 hours at room temperature.

5. Repeat the wash procedure described in step 3.

6. Use the plate immediately after blocking.

6. Standard Preparation

1. Prepare serially diluted standards immediately before use. Always prepare a fresh set of positive controls.

2. Reconstitute the protein standard by adding 100 µL ddH2O. Gently mix at room temperature for 10 minutes until completely in solution.

3. Aliquot unused reconstituted protein standard and store at -80°C. Refer to the vial label for protein quantity.

4. Label eight tubes Standards #1 through #8.

5. Prepare Standard #1 by diluting Stock Standard in 1X Blocking Buffer to the highest concentration specified in the product datasheet.

6. Prepare a seven-point standard curve using two-fold serial dilutions in 1X Blocking Buffer.

7. Prepare enough standard for duplicate readings. Each well requires 100 µL standard.

8. Use Standard #8 as the blank control with buffer only.

7. Assay Procedure

1. Equilibrate all materials and prepared reagents to room temperature.

2. Assay standards, controls, and samples in duplicate.

3. Dilute samples with 1X Blocking Buffer so the result falls within the assay dynamic range. If unknown, use a 1:2 dilution series.

4. Add 100 µL diluted standards and samples to each well. Seal and incubate for 1-2 hours at 37°C or room temperature on a plate shaker set to 400 rpm.

5. Wash the plate three times with 300 µL of 1X Wash Buffer and remove liquid completely after the final wash.

6. Add 100 µL diluted detector antibody to each well. Seal and incubate for 1 hour at 37°C or room temperature on a plate shaker set to 400 rpm.

7. Repeat the wash step.

8. For biotin detector workflows, add 100 µL diluted HRP-streptavidin solution to each well. Seal and incubate for 0.5 hour at 37°C or room temperature on a plate shaker set to 400 rpm. Omit this step for HRP-conjugated detector antibodies unless otherwise instructed.

9. Repeat the wash step.

10. Add 100 µL TMB substrate to each well and incubate for up to 10-20 minutes in the dark.

11. Optional: before adding Stop Solution.

12. Add 50 µL Stop Solution to each well.

13. Measure endpoint absorbance at 450 nm.

8. Calculations

- Calculate the average absorbance for the blank control standards.

- Subtract the average blank control absorbance from all other absorbance values.

- Plot blank-subtracted average absorbance for each standard concentration against target concentration.

- Use plate-reader or graphing software to fit the curve. A four-parameter logistic fit is often appropriate, but other fits may be evaluated.

- Interpolate sample concentrations from the standard curve and apply dilution factors.

9. Optimization Notes

- Optimize capture antibody concentration, detector antibody concentration, HRP reagent concentration, sample dilution, and substrate incubation time for the sample matrix and instrumentation.

- Generate a standard curve for every run.

- Use lot-specific vial labels and product documentation when they differ from example protocol values.