

How to use matched antibody pair kits for sandwich ELISA

A detailed protocol on how to use our matched antibody pair kits for sandwich ELISA.

Matched antibody pair kits include a capture and biotinylated detector antibody

Matched antibody pair and a calibrated protein standard. Kits are available in two sizes, with enough pair kits, reagents for either 2 or 10 x 96-well plates using a standard sandwich ELISA

Additional buffers and plates are required for the assay. An accessory pack can be purchased which includes all the reagents required to perform 10 x 96-well plates sandwich ELISA (ab210905).

Protocol summary

1. Add Capture Antibody diluted in coating buffer.
2. Incubate and then wash.
3. Block non-specific protein interactions by incubating with blocking buffer and then wash.
4. Add standards, controls and samples to the plate in duplicate.
5. Incubate the plate and then wash.
6. Add Detection Antibody. Incubate and then wash.
7. Add HRP Streptavidin solution for detection. Incubate and then wash.
8. Detect by adding TMB substrate.
9. Add Stop solution.
10. Measure plate on a microplate reader at 450 nm for endpoint and/or 600 nm kinetically.

Recommended reagents and consumables

- Matched Antibody Pair Kit ELISA Accessory Pack (10 x 96-well plates) (ab210905): containing Nunc™ MaxiSorp™ 96-well microplates, plate seals, Coating Buffer, 10X Wash Buffer, 10X Blocking Buffer, TMB and Stop Solution
- Nunc™ MaxiSorp™ 96-well plates (ab210903)
- Coating buffer 1X (ab210899) 35 mM NaHCO₃, 15 mM Na₂CO₃, pH 9.6 without NaOH
- Blocking buffer 10X (ab210904)

Dilute in PBS to 1X: 1% BSA*, 0.05% Tween® 20, in 1X PBS, pH7.2 - 7.4

*We recommend ≥96% purity BSA as less pure BSA can increase background

Note: 10X blocking buffer may contain precipitate. Mix well by vortex before use. Solution may be brought to room temperature to increase solubility. Precipitates have no negative effect on performance

- Wash Buffer 10X (ab206977)

Dilute in water to 1X: 0.05% Tween® 20 in 1X PBS

- TMB substrate (ab210902)
- Stop solution (ab210900): 4.9% orthophosphoric acid
- 10X Phosphate Buffered Saline (PBS) (ab128983)

Dilute in water to 1X: 0.14 M NaCl, 0.003 M KCl, 0.002 M KH₂PO₄, 0.01 M Na₂HPO₄

- Streptavidin-HRP Solution (ab210901), 50 µg/mL

Recommend diluting to 0.01-0.05 µg/mL in 1X Blocking Buffer

- Microcentrifuge tubes for dilution of standards
- Double distilled water (ddH₂O)
- Optional: Protease Inhibitor Cocktail (ab65621)
- Optional: BCA Protein Quantification Kit (ab102536)

Nunc™ MaxiSorp™ are unregistered trademarks owned by Thermo Fisher Scientific.

TWEEN® is a registered trademark of Croda International PLC

PROCLIN® is a registered trademark of The Dow Chemical Company or an affiliated company of Dow

Detailed protocol

1. Add 50 μL of 2 $\mu\text{g}/\text{mL}$ Capture antibody to each well of a 96-well of a high bind microplate (we recommend Nunc™ Maxisorp™ 96-well plate (ab210903)).
2. Seal the plate with a plate seal. Incubate the plate either overnight at 4°C or for 2 hours at room temperature on a plate rocker or shaker.
3. Wash plate three times with 350 μL of the recommended 1X Wash Buffer (ab206977). Remove liquid completely from last wash by tapping the plate vigorously against a pad of absorbent towels.
4. Reduce non-specific binding by adding 300 μL of 1X Blocking Buffer (ab210904) to each well, seal the plate and incubate either overnight at 4°C or for 2 hours at room temperature.
5. Repeat the wash procedure in step 2.
6. Plate can be used immediately after blocking or stabilized with Immunoassay 1X Blocking Buffer (ab171534) and stored at 4°C.
7. Prepare serially diluted standards immediately prior to use. Prepare enough of the standard solutions for duplicate measurements of each concentration.
8. Reconstitute the protein standard sample by adding 100 μL ddH₂O water. Gently mix at room temperature for 10 minutes to ensure that the protein is completely in solution. This is the Stock Standard Solution. Unused reconstituted protein standard should be aliquoted and stored -80°C.

Note: Refer to the vial label to see the quantity of protein standard provided.

9. Label eight tubes, Standards #1-8.
10. To prepare Standard #1, dilute the Stock Standard in 1X Blocking Buffer to the highest concentration specified in the product datasheet. A seven-point standard curve using 2-fold serial dilutions in 1X Blocking Buffer is recommended.

Note: Each well will require 50 μL of standard. Prepare enough standard dilutions to allow for duplicate readings.

Example of Stock standard and standard #1 preparation Label states that 10 ng protein standard is provided.

Reconstitute in 100 μL H₂O. Stock Standard concentration = 100 ng/mL

Product states required Standard #1 concentration should be 1 ng/mL

Dilute Stock standard 100X by adding 10 μL of Stock Standard to 1 mL of 1X Blocking Buffer for Standard #1

Create rest of the two-fold dilution series in 1X Blocking Buffer.

11. Standard #8 is the Blank control (buffer only) and contains no standard protein.
12. Dilute the experimental sample with 1X Blocking Buffer. Dilute the sample so that the resulting concentration is within the dynamic range of the assay. Multiple sample dilutions using 1:2 dilution series is advised if the concentration of the target protein is unknown.
13. Add 50 μ L diluted standard and samples to each well. Seal the plate and incubate for 2 hours at room temperature on a plate shaker set to 400 rpm.
14. Wash plate three times with 350 μ L of 1X Wash Buffer (ab206977). Remove liquid completely from last wash by tapping the plate vigorously against a pad of absorbent towels.
15. Dilute Detector Antibody from stock concentration of 0.25 mg/mL to the suggested working concentration of 0.5 μ g/mL in 1X Blocking Buffer (ab210904) or another appropriate diluent. Refer to the label to see the quantity of Detector Antibody provided.

Note: For best results, the concentration of detector antibody in the working solution may require optimization.

(See [ELISA troubleshooting tips](#) for more information.)

16. Repeat wash step as described as in step 14.
17. Add 50 μ L of diluted HRP-Streptavidin solution (ab210901) diluted in 1X Blocking buffer to each well. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
18. Repeat wash step as described in step 14.
19. Add 100 μ L of TMB Substrate to each well and incubate for up to 20 minutes in the dark on a plate shaker set to 400 rpm

Note: For best results, the incubation time requires optimization.

20. Add 100 μ L of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Plate can be read kinetically at 400 nm to monitor proper incubation time. (See [ELISA troubleshooting tips](#) for more information)
21. Measure the plate at 450 nm.
22. Analyze the data as described below.

Analyze the data

Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.

Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve. Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 5-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.

See our [webinar](#) for more information on calculating ELISA raw data.

See our [ELISA optimization guide](#) to design your own sandwich ELISA.