How to use Matched Antibody Pair Kit

For research use only - not intended for diagnostic use.

Precautions:

Some components in this kit contain ProClin® which may cause an allergic skin reaction or respiratory irritation.
The Stop Solution suggested for use with this kit is a concentrated acid solution and should be used with caution and adequate personal protective equipment (PPE).
The TMB substrate suggested for use with this kit may cause skin, eye, and respiratory irritation. Wear PPE. Wash hands thoroughly after handling. – Please review the SDS on the Abcam website prior to use.
Capture antibody contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Do not dispose the leftover component directly into sink.

Storage and Stability: Store kit at -20ºC immediately upon receipt.

Limitations:

The supplied kit is intended for research use only. Not for use in diagnostic procedures.
This kit contains sufficient materials to perform multiple sandwich ELISA, provided the following conditions are met:

- The reagents are prepared as described in the booklet.
- The assay is run as described in the Assay Procedure.
- The recommended reagents and solutions are used.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Recommended reagent, solution 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.
The reagent and solutions listed above may also be purchased separately:
– Nunc™ MaxiSorp™ 96-well plates (ab210903)
– Coating buffer 1X (ab210899): 35 mM $NaHCO_3$, 15 mM Na_2CO_3, pH 9.6
– Blocking buffer 10X (ab210904): Dilute in PBS to 1X: 1% BSA*, 0.05% Tween® 20, in 1X PBS, pH 7.2 - 7.4 *We recommend ≥96% purity BSA as less pure BSA can increase background
– 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 KH_2PO_4, 0.01 M Na_2HPO_4
– 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)

Required Equipment:

– Microplate reader capable of measuring absorbance at 450 nm.
– Multi-channel and single-channel pipettes.

Recommended Equipment:

– Plate shaker or rocker.

Reagent Preparation

Equilibrate all reagents to room temperature (18-25ºC) prior to use.
Allow all reagents to sit for a minimum of 15 minutes with gentle shaking after the initial reconstitution. Mix well before use.
Dilute reagents to 1X working concentrations, if required before use.
Reagent dilutions should be prepared and used immediately.
Prepare only as much reagents as is needed on the day of the experiment.
Antibody buffer information: The Capture antibody is provided in a glycerol free formulation and the Detector antibody is provided in a buffer that contains glycerol.

Capture Antibody: Capture Antibody is provided at 1 mg/mL. Dilute the Capture Antibody to the suggested working concentration of 2 µg/mL in Coating Buffer (ab210899). Add 50 µL per well.
∆ Note: For best results in your application, optimization of the concentration of capture antibody in Coating Buffer may be required.

Detector Antibody: Detector Antibody is provided at 0.25 mg/mL. Dilute the biotin-labelled Detector Antibody to the suggested working concentration of 0.5 µg/mL in Blocking Buffer or other appropriate diluent. Add 50 µL per well.
∆Note: For best results in your application, optimization of the concentration of detector antibody in 1X Blocking Buffer may be required.

Plate Preparation

1) Add 50 µL of 2 µg/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 4ºC 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 4ºC or for 2 hours at room temperature.
5) Repeat the wash procedure in step as described in step 3.
6) Plate should be used immediately after blocking.

Standard Preparation

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of positive controls for every use. The following section describes the preparation of a two-fold diluted standard curve for duplicate measurements (recommended).

1) 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 at -80°C. Δ Note: Refer to the vial label to see the quantity of protein standard provided.
2) Label eight tubes, Standards #1 through #8
3) To prepare Standard #1, dilute an aliquot of 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.

   Δ 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 (i.e. 1000 pg/mL). Dilute Stock standard 100X by adding 10 µL of Stock Standard to 990 µL of 1X Blocking Buffer for Standard #1. Create rest of the two-fold dilution series in 1X Blocking Buffer.
4) Standard #8 is the Blank control (buffer only) and contains no standard protein.

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate
Prepare all reagents and standards as directed in the previous sections

1) 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.
2) Add 50 µL 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.
3) Wash plate three times with 350 µL of 1X Wash Buffer (ab206977). Remove liquid completely from last wash by tapping the plate vigorously against a pad of absorbent towels.
4) Dilute Detector Antibody from stock concentration of 0.25 mg/mL to the suggested working concertation of 0.5 µg/mL in 1X Blocking Buffer (ab210904) or other appropriate diluent.
5) Add 50 µL of diluted Detector antibody to each well. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

   Δ Note: For best results, the concentration of detector antibody in the working solution may require optimization.
   (See https://www.abcam.com/en-us/technical-resources/applications/elisa/troubleshooting for more info.)
6) Repeat wash step as described as in step 3.
7) Dilute HRP-Streptavidin solution (ab210901) to 0.05 µg/mL in 1X Blocking buffer. Add 50 µL of diluted HRP-Streptavidin solution to each well. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm. Δ Note: For best results, the concentration of diluted HRP streptavidin solution may require optimization.
8) Repeat wash step as described in step 3.
9) Add 100 µL of TMB Substrate to each well and incubate for up to 20 minutes in the dark on a plate shaker set to 400 rpm.

   Δ Note: For best results, the incubation time requires optimization.
10) Before adding Stop Solution, plate can be read kinetically at 600nm to monitor proper incubation time.
11) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker at 400 nm for 1 minute to mix. (See https://www.abcam.com/en-us/technical-resources/applications/elisa/troubleshooting for more information)
12) Measure the endpoint of the plate at 450 nm.
13) Analyze the data as described below.

Calculations

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, semilog, 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 https://www.abcam.com/kits/calculate-accurate-protein-concentrations for more information on calculating ELISA raw data.
See our ELISA optimization guide: https://www.abcam.com/en-us/technical-resources/applications/elisa/troubleshooting to design your own sandwich ELISA.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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