Sandwich ELISA Protocol

General procedure and tips for sandwich ELISA including details of how to find matched pair antibodies.

Western Blotting

A sandwich ELISA measures the amount of antigen between two layers of antibodies (capture and detection antibody). The antigen to be measured must contain at least two antigenic sites capable of binding to antibody, since at least two antibodies act in the sandwich.

Either monoclonal or polyclonal antibodies can be used as the capture and detection antibodies in sandwich ELISA systems. Monoclonal antibodies recognize a single epitope that allows fine detection and quantification of small differences in antigen. A polyclonal is often used as the capture antibody to pull down as much of the antigen as possible.

The advantage of sandwich ELISA is that the sample does not have to be purified before analysis, and the assay can be very sensitive (up to 2 to 5 times more sensitive than direct or indirect).

General note:

Sandwich ELISA procedures can be difficult to optimize and tested match-paired antibodies should be used. This ensures the antibodies are detecting different epitopes on the target protein so they do not interfere with the other antibody binding. Therefore, we are unable to guarantee our antibodies in sandwich ELISA unless they have been specifically tested for sandwich ELISA.

Please review antibody datasheets for information on tested applications.

General procedure

Coating with capture antibody

1. Coat the wells of a PVC microtiter plate with the capture antibody at a concentration of 1-10 µg/ml in carbonate/bicarbonate buffer (pH9.6). Cover the plate with an adhesive plastic and incubate overnight at 4° C.

If an unpurified antibody is used (e.g. ascites fluid or antiserum), you may need to compensate for the lower amount of specific antibody by increasing the concentration of the sample protein (try 10 µg/ml).

2. Remove the coating solution and wash the plate twice by filling the wells with 200 µl PBS. The solutions or washes are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

Blocking and adding samples

3. Block the remaining protein-binding sites in the coated wells by adding 200 µl blocking buffer, 5% non-fat dry milk/PBS, per well.

4. Cover the plate with an adhesive plastic and incubate for at least 1-2 hours at room temperature or, if more convenient, overnight at 4°C.

5. Add 100 µl of appropriately diluted samples to each well. For accurate quantitative results, always compare signal of unknown samples against those of a standard curve. Standards (duplicates or triplicates) and blank must be run with each plate to ensure accuracy. Incubate for 90 min at 37°C.

For quantification, the concentration of the standard used should span the most dynamic detection range of antibody binding. You may need to optimize the concentration range to ensure you obtain a suitable standard curve. For accurate quantitation, always run samples and standard in duplicate or triplicate.

For research purposes only ! Not for therapeutic or diagnostic purposes in humans or animals !

Add: Room 703, Building 6, No. 333 Guiping Rd., Xuhui District, Shanghai City, P.R. China 200233
Tel/ Fax: +86 021-54651191 **Product Inquiry:** market@yhsbio.com
Placing Orders: order@yhsbio.com **Technical Support:** support@yhsbio.com
www.yhsbio.com

6. Remove the samples and wash the plate twice by filling the wells with 200 µl PBS.

Incubation with detection antibody and then secondary antibody

7. Add 100 µl of diluted detection antibody to each well.

Ensure the secondary detection antibody recognizes a different epitope on the target protein than the coating antibody. This prevents interference with the antibody binding and ensures the epitope for the second antibody is available for binding. Use a tested matched pair whenever possible.

8. Cover the plate with an adhesive plastic and incubate for 2 hours at room temperature.

9. Wash the plate four times with PBS.

10. Add 100 µl of secondary antibody conjugated, diluted at the optimal concentration (according to the manufacturer) in blocking buffer immediately before use.

11. Cover the plate with an adhesive plastic and incubate for 1-2 hours at room temperature.

12. Wash the plate four times with PBS.

Detection

Although many different types of enzymes have been used for detection, horse radish peroxidase (HRP) and alkaline phosphatase (ALP) are the two most widely used enzymes employed in ELISA assay. It is important to consider the fact that some biological materials have high levels of endogenous enzyme activity (such as high ALP in alveolar cells, high peroxidase in red blood cells) and this may result in a nonspecific signal. If necessary, perform an additional blocking treatment with Levamisol (for ALP) or with 0.3% solution of H_2O_2 in methanol (for peroxidase).

ALP substrate

For most applications, pNPP (p-Nitrophenyl-phosphate) is the most widely used substrate.The yellow color of nitrophenol can be measured at 405 nm after 15-30 min incubation at room temperature. This reaction can be stopped by adding equal volume of 0.75 M NaOH.

HRP chromogenes

The substrate for HRP is hydrogen peroxide. Cleavage of hydrogen peroxide is coupled to oxidation of a hydrogen donor which changes color during reaction.

TMB (3,3',5,5'-tetramethylbenzidine)

Add TMB solution to each well, incubate for 15-30 min, add equal volume of stopping solution (2 M H_2SO_4) and read the optical density at 450 nm.

OPD (o-phenylenediamine dihydrochloride)

The end product is measured at 492 nm. Be aware that the substrate is light sensitive so keep and store it in the dark.

ABTS (2,2'-azino-di-[3-ethyl-benzothiazoline-6 sulfonic acid] diammonium salt)

The end product is green and the optical density can be measured at 416 nm.

Some enzyme substrates are considered hazardous (potential carcinogens), therefore always handle with care and wear gloves.

13. Dispense 100 µl (or 50 µl) of the substrate solution per well with a multichannel pipet or a multipipet.

For research purposes only ! Not for therapeutic or diagnostic purposes in humans or animals !

Add: Room 703, Building 6, No. 333 Guiping Rd., Xuhui District, Shanghai City, P.R. China 200233
Tel/ Fax: +86 021-54651191 **Product Inquiry:** market@yhsbio.com
Placing Orders: order@yhsbio.com **Technical Support:** support@yhsbio.com
www.yhsbio.com