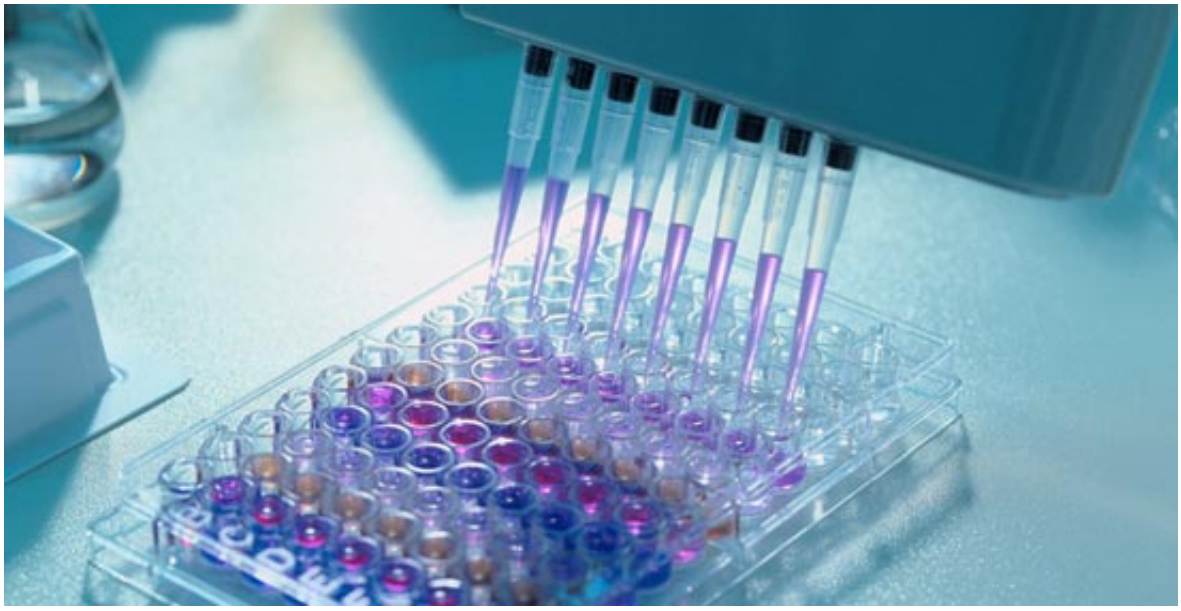
Sandwich ELISA

Author : Dan Souw



Sandwich ELISA

This is the third post in a series on ELISA, and this one will focus on the Sandwich ELISA.

A sandwich ELISA differs from the direct and indirect ELISAs previous types we've written about in one key aspect: a capture antibody.

This type of assay, as the name indicates, sandwiches an analyte between two layers of antibody—a capture and a detection antibody.

The capture antibody is used to pull out your particular analyte of interest from your sample solutions, be it plasma, serum or cell culture. Whether to use a monoclonal or a polyclonal antibody for capture will depend on your assay but, generally, the wide variety of epitopes that an affinity purified polyclonal antibody binds to tends to pull more of your analyte out of solution. This may be especially true if your target analyte binds other proteins.

However, if your assay is to be used for a long period of time and requires consistency, then a monoclonal may be more suitable, the trade-off being that you may have to have longer incubation periods.

The same applies for the detection antibody, but with the caveat that it is generally better to use a

monoclonal detection if your capture is polyclonal, or a polyclonal detection if your capture is monoclonal. The reason for this is to help keep background binding to a minimum.

In a sandwich ELISA your detection antibody may be directly labelled in some way, such as with biotin, or covalently linked to a reporter enzyme of some kind. You can also use a secondary antibody to detect your detection antibody—an indirect sandwich ELISA—but generally a labelled detection antibody is best.

An advantage of the Sandwich ELISA is that they have high specificity, can be used with complex samples since, generally, you do not need to purify your sample beforehand, and they can be very sensitive, more-so than direct or indirect ELISAs. In some cases, when coupled with the right detection system, down to the picogram level.

They also have the advantage of being quite straightforward to perform. On the flip-side, they can be very difficult to develop from scratch.

With that in mind, below you will find a basic protocol for a sandwich ELISA, with the usual qualification that the specific concentrations of capture and detection antibodies you might need to use will need to be optimised for your analyte and reagents, but this guide should get you on the right track.

Reagents:

96-well microplate

Coating buffer: PBS (pH 7.4) or carbonate/bicarbonate (pH9.6) for coating

Washing buffer: PBS + 0.05% Tween-20

Blocking buffer: PBS + 1% BSA

General tip: If adding the same reagent *to all wells of a plate, a multichannel pipette can save a huge amount of time.*

Plate preparation

1. Dilute the capture antibody to the manufacturers recommended concentration to make about 10 mL of coating

Antibodies are typically diluted either in PBS or carbonate/bicarbonate buffer. Follow the manufacturer's instructions.

Note: avoid using anything containing BSA/protein, as this will compete with your capture antibody for binding in the plates microwells.

2. Add 100 ?L per well of coating solution to the plate.
3. Cover with a plate sealer and incubate overnight at +4 °C.
4. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes.

Tip: rap the plate smartly on some paper towels after washing to ensure all washing buffer is removed.

5. Add 300 ?L of blocking buffer to all wells of the plate and incubate at room temp for at least an hour. Ideally 2-3 hours.

This step uses the BSA in solution to bind to any remaining free binding sites in each microwell, 'blocking' the detection antibodies, antigen or other samples components from binding non-specifically to the plate.

Note: While the plate is blocking, remove samples to thaw and prepare any calibration standards or QC samples and diluted samples (if needed).

Ideally, samples, standards and QC samples (e.g. a high, medium and low) will be added in duplicate to the plate.

Also, this is a great time to prepare a plate plan, so you know which samples should go where. Preparing and following a plate plan ahead of time will save you a lot of trouble.

Assay procedure

1. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes, as before.
2. Add 100 ?L of samples, standards, QC samples (if using) and samples to each well of the

plate *according to the plate plan*.

Tip: Be sure to use a buffer blank/ zero standard as well. This can then be subtracted from all other well results to help correct small levels of background signal.

3. Cover with a plate sealer and incubate for 2 hours at room temperature. Gentle agitation on a plate shaker can be used.
4. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes, as before.
5. Add 100 ?L of detection antibody (diluted according to the manufacturer's instructions) to each well of the plate.
6. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes, as before.
7. Add 200 ?L of substrate solution to each well and incubate for 20 minutes at room temperature. After 20 minutes, add stop solution, if required. (see below).

Note: The substrate will depend on your detection system/antibody. This may entail an additional step, such as incubating the detection antibody with strep-HRP before adding the detection solution. If using HRP, most assays use TMB as the substrate, in which case 50 ?L of 0.16M sulfuric acid would be added to each well.

8. Immediate use a plate reader (set to 450 nm minus 570 nm, for wavelength correction, for TMB) to determine the optical density (OD) of each well.

Calculation of results

Chances are, if you have a plate reader, you have some software to interpret results.

Generally, this will involve calculating the mean absorbance for each set of calibration standards, controls and samples, and subtracting the blank value.

Use the values from the standards to make a calibration curve, plotting concentration (x-axis) against OD (y-axis). Most commonly, and especially so for sandwich ELISA, a four or five parameter logistic curve fit (4-PL or 5-PL) is usually best.

Use this curve to interpolate sample concentrations. Don't forget to multiply the results of any

diluted samples read from the calibration curve by the dilution factor to get the ‘original’ concentration.

We hope this general protocol will be useful. As ever, specifics will depend on the reagents, samples and antigen of interest, so where possible follow the manufacturer's guidance.

We may cover tips for developing sandwich ELISAs in a future post. If you’re interested, let us know!