Direct ELISA

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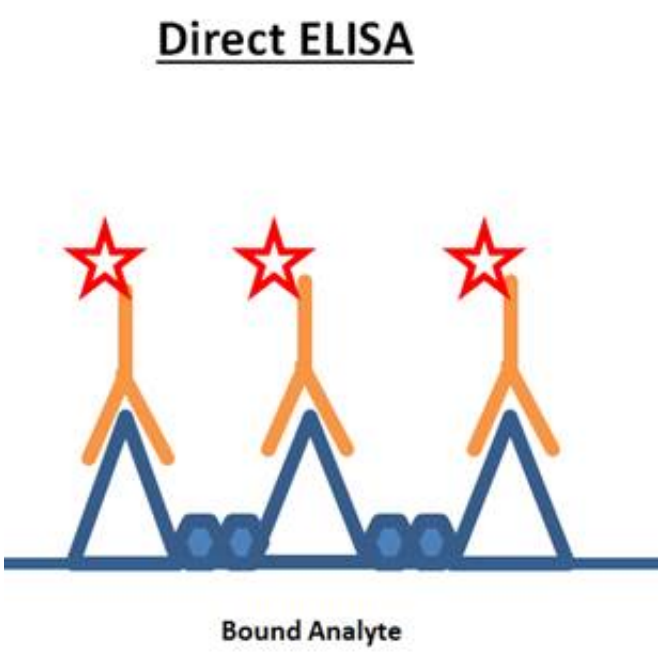

Direct ELISA

This is the second in a series of posts and protocols on Enzyme Linked Immunosorbent Assays (ELISAs). This one deals with the ‘Direct ELISA’.

Background

A Direct ELISA is the simplest form of ELISA. The analyte of interest is coated/immobilised on to a microtitre plate, then an enzyme conjugated antibody against that analyte is added to each well of the plate, binding to the analyte, followed by a substrate for the enzyme which develops a signal in direct proportion to the amount of antibody bound.

The process is basically the same as immunohistochemical staining.

Based on this simple description it may be hard to imagine what the use of a direct ELISA might be. It’s true they’re not common, but they do service particular niches.

For example, Direct ELISAs are often used to test the efficiency or reactivity of new antibodies against an analyte, or to determine the concentration of a purified protein against a set of known

standards.

A direct ELISA can be used qualitatively (i.e. to give a yes/no answer to the question ‘is there any analyte in this sample?’), or quantitatively (i.e. comparing a sample to a series of calibrators and interpolating their concentration from the resulting calibration curve).

The main advantages of a direct ELISA is that, with so few steps, they are very quick to do. The lack of complexity also eliminates any potential problems with cross-reactivity of secondary antibodies.

With that in mind, here is a basic protocol for performing a Direct ELISA – again, the specific concentrations you might need to use will need to be optimised for your analyte/antibody pair, but this guide should get you on the right track.

Whether you want to titrate the analyte or the antibody to give a quantitative response will depend on what your intention with the assay is.

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.*

Direct ELISA Protocol

Plate preparation

1. Dilute the analyte to a final concentration of 20 ?g/mL in coating buffer and add 50 ?L to each well of a microtitre plate.

Note: If your assay is comparing unknown concentrations of the analyte *to known concentrations of analyte, then at this step you would want to prepare a dilution series of you analyte, for example from 40 ?g/mL, serially diluted 1 in 2 with coating buffer.*

2. Cover with a plate sealer and incubate overnight at +4 °C. Alternatively, incubate for 2 hours at room temperature.
3. 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.

Blocking

4. Add 200 ?L of blocking buffer to all wells of the plate, cover with a plate sealer 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.

5. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes.

Incubation with antibody

6. Add 100 ?L per well of the conjugated detection antibody prepared at a concentration according to the manufacturer's instructions (this could vary greatly).

Note: If your assay is comparing unknown concentrations of the antibody *to a standard or reference antibody, then at this step you may want to prepare a dilution series of your reference antibody or sample.*

7. Cover with a plate sealer and incubate for 2 hours at room temperature.

Note: This step may need optimisation. Generally, 2 hours should be enough to see some kind of signal, but in the event of weak signal you may even need to incubate overnight at +4 °C.

8. Aspirate each well, and wash with 200 ?L of wash buffer. Repeat twice more for a total of three washes.

Detection

9. Add 100 ?L of substrate solution to each well and incubate for 20 minutes at room temperature. After 20 minutes, or sufficient color development, 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.

10. Immediately 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.

Data Analysis

How you perform this will very much depend on what you are trying to measure. If it is a qualitative assay, then a simple spread sheet can be used to give you a ‘+ve’ or ‘-ve’ result for sample wells based on their OD. If quantitative.

If your assay is quantitative, use the values from the standards to make a calibration curve, plotting concentration (x-axis) against OD (y-axis). Most commonly, a four parameter logistic curve fit (4-PL) can be used, though sometimes with a direct ELISA a straight line fit performed in a spreadsheet may work equally well.

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 Direct ELISA protocol will be useful. As ever, specifics will depend on the reagents, samples and antigen of interest, so where possible follow the manufacturers guidance.