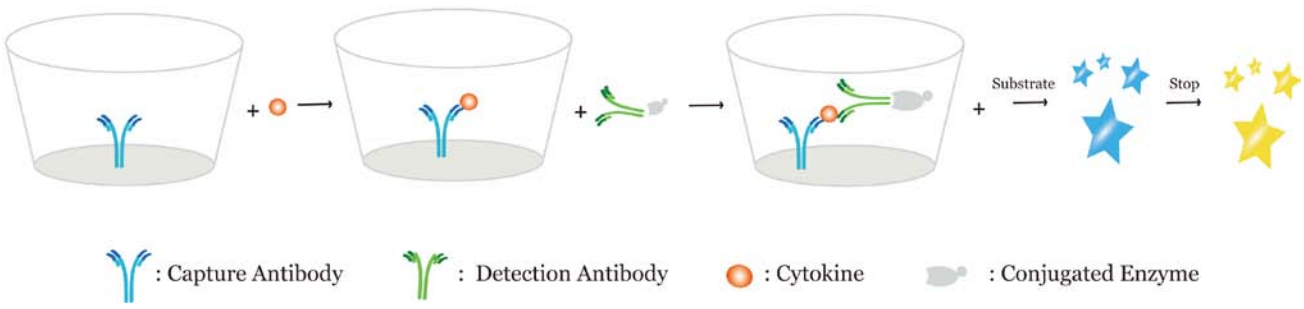
WEBSITE www.immunoway.com
E-MAIL order@ immunoway.com
tech@ immunoway.com

SANDWICH ELISA PROTOCOL

The Sandwich ELISA measures the amount of antigen between two layers of antibodies (i.e. 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.

Note: If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.



General Procedure:

1. Addition of Known Standard and Unknown Sample to Immunoassay

Prior to applying an unknown sample to the Sandwich ELISA, the immunoassay must be performed using a serial dilution of a known standard sample in order to determine the standard curve. This is necessary to allow for the interpretation of results generated by the unknown samples.

a) Dilute the known standard sample in a series of microfuge tubes (Details according to each product data sheet). Mix each tube thoroughly by inverting several times or by vortexing. Add 100 µl of each serial dilution step into the wells of a specified row or column of the 96-well microtiter plate in duplicate or triplicate. **Note:** Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom. If a standard curve has already been generated, substitute the standard with the unknown sample of interest.

b) Afterwards, seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available. When the plate is completely sealed, incubate at room temperature for 2 hours.

2. Application of Detection Antibody to Capture Antibody-Bound Samples

a) Aspirate the standard and unknown solutions out of the microplate wells. If your lab does not have a vacuum-based aspirator, you may dump the solutions from the microplate into a waste container and blot 3-4 times on a stack of paper towels until most or all of the liquid is removed from the wells. Add 300-400 µl of Wash Buffer to each well being used and gently shake for 5-7 minutes on an orbital shaker. Perform this wash step 4 times consecutively.

b) After the wash step, dilute the detection antibody solution. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Ensure that there is enough diluted detection antibody solution for all wells being used. Add 100 µl of the diluted detection antibody solution into each well and incubate at room temperature for 2 hours.

3. Conjugation of Avidin-Horseradish Peroxidase Enzyme with Detection Antibody

a) Remove the detection antibody solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash.

b) After the 4th wash step, add 100 µl of Ready-to-Use Avidin-HRP Conjugate Solution into each well and incubate at room temperature for 30 minutes.

4. Application of Liquid Substrate for Colorimetric Reaction

a) Remove the Avidin-HRP conjugate solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Prepare the TMB substrate solution by bringing it to room temperature without exposure to fluorescent or UV light as these may degrade the TMB. Perform 4 consecutive wash steps with gentle shaking between each wash.

b) After the 4th wash step, add 100 µl of TMB substrate solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue very quickly depending on analyte and/or detection antibody-HRP concentrations. Once the blue color has ceased to develop further, immediately add 100 µl of Stop Solution to each well being used. The color in the wells should immediately change from blue to yellow.

c) The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the Stop Solution, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. **Caution:** Readings made directly at 450 nm without correction may be higher and less accurate.

5. Generation of Standard Curve and Interpretation of Data

a) Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.

b) Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis. Note: Shown on the next page is an example of typical data produced by analysis of the standard sample.