LP108 – Sandwich ELISA

The following is a general protocol for a Sandwich ELISA using matched pair antibodies. The precise conditions should be optimized for a particular assay. Expected OD490 readings are between 0.7 and 1.3 for a substrate development time of 20-30 minutes.

Materials

Multi-pipette reservoirs
1.5-ml microcentrifuge tubes
96-well Nunc Immuno MaxiSorp microtiter plate

Solutions

10 X TBS: 500 mM Tris, 1M NaCl pH7.4

1 X TBS: 50 ml 10 x TBS, fill to 500 ml with distilled water. pH7.4

Coating Solution: 50 mM carbonate buffer, pH 9.6

Blocking Solution: 1% RIA grade BSA (Sigma – A7888) in 1 X TBS +0.05% TWEEN 20

Capture/Detection Antibody Solution: 1X Blocking Solution

Wash Solution: 1X TBS + 0.05% TWEEN 20 (Use 1 X PBS +0.05% TWEEN 20 if PBS used as a coating solution)

Citrate Buffer: 121.5 ml of 0.1M Citric Acid, pH to 5.0 using 128.5 ml 0.2M dibasic sodium phosphate. Fill to 500 ml with distilled water and check pH.

HRP Substrate Buffer: Prepare 10 ml HRP Substrate Buffer by mixing 9 ml citrate buffer, 1 ml 4 mg/ml OPD. Add 4 µl H_2O_2 immediately before use.

Stop Solution: 4 N H2SO4

Protocol

1. Dilute the capture antibody to the appropriate concentration in coating buffer and coat plate with 100 µl per well.
2. Cover with sealing film and incubate at 4°C overnight.
3. Empty plate and tap out residual liquid.
4. Wash twice with 200 µl Wash Solution.
5. Add 200 µl Blocking Solution to each well. Incubate 2 hours at room temperature.
6. Empty plate and tap out residual liquid.
7. Wash twice with 200 µl Wash Solution.

8. Add 100 µl standard/sample diluted in 1X blocking solution to each well. Incubate 2 hours at room temperature.
9. Empty plate, tap out residual liquid.
10. Wash with 200 µl Wash Solution. Invert plate to empty, tap out residual liquid. Repeat 3 times.
11. Add 100 µl diluted HRP-conjugated detection antibody to each well. Incubate 2 hour at room temperature in the dark.
12. Empty plate, tap out residual liquid and wash as described in step 10.
13. Dispense 50 µl HRP Substrate Solution per well. Develop the color for 2-30 minutes at room temperature in the dark.
14. Stop color development by adding 50 µl of Stop Solution per well and immediately read at 492 nm in plate reader.

Assay Optimization

To determine the optimal capture and detection antibody concentration perform a grid experiment using one plate. For a monoclonal capture antibody, start in the range of 0.5 – 8 µg/ml, for polyclonal use 0.2 – 1 µg/ml. For the detection antibody, use concentrations in the range of 0.25 – 2 µg/ml for monoclonal and 50 – 400 ng/ml for polyclonal. Check datasheet for recommended starting concentrations.

Divide a 96 well ELISA plate into 4 quadrants and use a different detection antibody in each quadrant. Each row within the quadrant represents a different point on the standard curve and each column is a different capture antibody concentration in duplicate (see diagram below for example of plate set up).

50 ng/ml detection

100 ng/ml detection

	1µg/ml capture	1µg/ml capture	2µg/ml capture	2µg/ml capture	4µg/ml capture	4µg/ml capture	1µg/ml capture	1µg/ml capture	2µg/ml capture	2µg/ml capture	4µg/ml capture	4µg/ml capture
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard
C	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard
D	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard
E	0	0	0	0	0	0	0	0	0	0	0	0
F	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard	1ng/ml standard
G	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard	2ng/ml standard
H	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard	4ng/ml standard

200 ng/ml detection

400 ng/ml detection