biossusa.com
800.501.7654
+1.781.569.5821
support@biossusa.com

SANDWICH ELISA PROTOCOL

This protocol is a recommendation only. Please optimize the procedure since experimental conditions can vary for different tissue samples.

COATING WITH CAPTURE ANTIBODY

1. Coat the wells of a 96-well plate with 100uL of the capture antibody diluted in bicarbonate/carbonate solution.
2. Cover the plate with parafilm or plastic adhesive and incubate overnight at 4°C.
3. Remove the coating solution and wash plate 2 times with 200uL PBS +0.05% Tween20 (PBST). The solutions and washes are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel or by aspiration.

BLOCKING

4. Block the remaining protein-binding sites in the coated wells by adding 200uL blocking buffer per well.
5. Cover the plate and incubate for 2 hours at room temperature.
6. Wash the plate 2 times with 200uL PBST.

ADDING SAMPLES

7. Add 100uL of appropriately diluted samples and standards.
   Note: For accurate quantitative results, always compare signal of unknown samples against those of a standard curve. Standards (in duplicate and triplicate) and a blank must be run with each plate for analysis and to ensure accuracy.
8. Cover the plate and incubate for 2 hours at room temperature.
9. Wash the plate 2 times with 200uL PBST.

INCUBATION WITH DETECTION ANTIBODY

10. Add 100uL of diluted detection antibody to each well.
    Note: Be sure to check that the detection antibody recognizes a different epitope on the target protein to the capture antibody. This prevents interference with antibody binding.
10. Cover the plate and incubate for 2 hours at room temperature.
12. Wash the plate 4 times with PBS.
13. Add 100uL of conjugated secondary antibody, diluted in blocking buffer immediately before use.
14. Cover the plate and incubate for 2 hours at room temperature.
15. Wash the plate 4 times with PBS.

DETECTION

16. Add 100uL of the substrate solution per well.
17. After sufficient color development add 100uL of stop solution to the wells.
18. Read the absorbance of each well with a plate reader.
19. Prepare a standard curve from the data produced from the serial dilutions with concentration on the X-axis vs. absorbance on the Y-axis. Interpolate the concentration of the sample from this standard curve.