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INDIRECT ELISA PROTOCOL

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

COATING ANTIGEN TO PLATE

1. Coat the wells of a 96 well plate with 100uL of the desired antigen diluted in bicarbonate/carbonate solution. Include a serial dilution of the antigen for analysis.
2. Cover plate with parafilm or plastic adhesive and incubate overnight at 4°C.
3. Remove coating solution and wash plate 2 times with 200uL PBS+0.05% Tween20. The solutions or washed are removed by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel.

BLOCKING

4. Block the remaining protein binding sites in the coated wells by adding 200uL of blocking buffer(1% milk/PBS or 1%BSA/PBS).
5. Cover the plate and incubate for 2 hours at room temperature.
6. Wash plate 2 times with 200uL PBST.

ANTIBODY INCUBATION

7. Add 100uL of antibody diluted in blocking buffer.
8. Cover the plate and incubate for 2 hours at room temperature.
9. Wash plate 2 times with 200uL PBST.
10. Add 100uL of conjugated secondary antibody diluted in blocking buffer.
11. Cover the plate and incubate for 2 hours at room temperature.
12. Wash plate 2 times with 200uL PBST.

DETECTION

13. Add 100uL of the substrate solution per well.
14. After sufficient color development add 100uL of stop solution to the wells.
15. Read the absorbance of each well with a plate reader.

ANALYSIS

16. 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.