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Test-serology-protocol

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Protocol status: In development
We are still developing and optimizing this protocol

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Abstract

This is an assay describing indirect sandwich ELISA for RDP Ag of SARS-CoV-2.
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Guidelines

Materials

Hardware

Clear 96 well plate

Multi-channel precision pipettor with disposable plastic tips

Plate reader or luminometer equipped to detect the substrate

	Reagents Coating buffer:	0.2 M sodium carbonate/bicarbonate, pH 9.4
	Capture antibody:	Diluted in Coating Buffer (see Appendix for appropriate concentration range).
	Wash buffer:	0.1 M phosphate, 0.15 M sodium chloride, pH 7.2 containing 0.05 % Tween 20

	Note: 0.1M Phosphate can be replaced by 25 mM Tris, pH 7.2	
	Blocking buffer:	2% (w/v) Bovine Serum Albumin (BSA) in Wash Buffer
	Note: alternative buffers are listed in the product list at the end of this document	
	Standard diluent:	2% (w/v) BSA in Wash Buffer. Note: Ideally the standard diluent composition would be as close as

possible to the sample matrix. For example if measuring the concentration of an antigen in culture supernatant, culture medium should be used as the standard diluent. However biological sample matrices such as serum are impossible to replicate, therefore BSA is commonly used in these instan

		ces. Often the blocking buffer is also used as the standard diluent
	Samples/standards:	See preparation sections below.
	Detection antibody (biotinylated):	Diluted in 1/5 strength standard diluent (see Appendix for appropriate concentration range)
	Enzyme conjugate:	Streptavidin-HRP diluted in 1/5 strength standard diluent (see Appendix for appropriate conc

		entration)
	Substrate:	TMB substrate (see product list for a list of alternative substrates)
	Stop solution:	2M sulfuric acid

A.Preparation of Standards

1 Typically a standard curve may span concentrations from 0 to 1000 pg/ml but may go as high as 3000 pg/ml depending on the predicted amount of antigen in the sample and the amount of standard protein available. Typically two-fold or three-fold dilutions of the standard protein are prepared from the stock solution using the standard diluent. When preparing serial dilutions of a protein standard, use fresh tips after each dilution.

B. Preperation of Samples

2 If the concentration of antigen in the sample potentially exceeds the highest point of the standard curve (i.e. > 1,000 pg/ml) prepare one or more dilutions of the sample using the standard diluent.

C. Procedure

3 **Important:** Do not allow the plate to dry at any point

4 Dilute the capture antibody to the appropriate concentration allowing sufficient volume for 50-100 ul per well.

5 Add the diluted capture antibody to the plate, cover and incubate for 2 hours at room temperature (RT).

6 Remove the solution and wash the plate with 200 ul per well wash buffer for 3 × 5 minutes on a shaking platform.

7 Add 300 ul blocking buffer per well, cover the plate and incubate for 1 hour at room temperature. Alternatively block overnight at 4uC.

8 Prepare the samples and standards. The volume per well should be the same as the capture antibody used in step 1.

9 Remove the blocking buffer and add the samples and standards. Cover the plate and incubate for 1 hour at RT.

10 Dilute the biotinylated detection antibody to the appropriate concentration. The volume per well should be the same as the capture antibody used in step 1.

11 Remove the solution and wash the plate with 200 µl per well wash buffer for 3 × 5 minutes on a haking platform.

12 Add the diluted detection antibody to the plate, cover and incubate for 1 hour at RT.

13 Remove the solution and wash the plate with 200 µl per well wash buffer for 3 × 5 minutes on a shaking platform.

14 Dilute the enzyme conjugate to the appropriate concentration. The volume per well should be the ame as the capture antibody used in step 1.

15 Add the diluted enzyme conjugate to the plate, cover and incubate for 1 hour at RT.

16 Remove the solution and wash the plate with 200 µl per well wash buffer for 6 × 5 minutes on a shaking platform

17 Add substrate solution to the plate. The volume per well should be the same as the capture antibody used in step 1.

18 Incubate the plate at RT until the desired color intensity is reached. Ideally a clear gradient will result for the standards.

19 Stop the reaction by adding an equal amount of stop solution.

20 If using TMB, measure the absorbance at 450 nm. For other substrates use the appropriate detection technique.