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SARS-CoV-2 Antigen Detection ELISA

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1 Works for me dx.doi.org/10.17504/protocols.io.bk2ckyaw

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SARS-CoV-2

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MATERIALS

NAME	CATALOG #	VENDOR
Tween 20	P7949	Sigma Aldrich
Phosphate Buffered Saline (25x) pH 7.6	PBS-20000	ScyTek
Sodium Bicarbonate	S6297	Sigma Aldrich
Hydrochloric Acid	A142-212	Fischer Scientific
BSA Sigma #A3059	A3059	Sigma Aldrich
Blocker™ Casein in PBS Thermo Fisher Scientific #37528	37528	Thermo Fisher Scientific
ELAST® ELISA Amplification System Perkin Elmer #NEP116E001EA	NEP116E001EA	Perkin Elmer
HRP Conjugation Kit - Lightning-Link Abcam #ab102890	ab102890	Abcam
SureBlue Reserve TMB 1-Component Microwell Peroxidase Substrate KPL Cat No. 53-00-03	53-00-03	KPL
Deionized (DI) Water (dan use lab DI water)		
Purified recombinant capture and detection antibodies (see Table under Materials Text)		

MATERIALS TEXT

- Purified recombinant capture and detection antibodies:

Antibody	Binding domain*	Species	Isotype	Description
DH1219	NP	Human	IgG1	NP capture
HRP-DH1218	NP	Human	IgG1	NP detection
DH1041	Spike	Human	IgG1	Spike capture
HRP-DH1046	Spike	Human	IgG1	Spike detection

**SARS-CoV-2 Spike (S) and Nucleocapsid/nucleoprotein (NP)*

Reagents

1
- Tween 20, Sigma Cat. No. P7949
- Phosphate Buffered Saline (25x) pH 7.6 from ScyTek Laboratories, Cat No: PBS-20000
- Sodium Bicarbonate, Sigma, Cat No: S6297
- Hydrochloric Acid, Fischer Scientific Cat No. A142-212
- BSA Sigma #A3059
- Blocker™ Casein in PBS Thermo Fisher Scientific # 37528
- ELAST® ELISA Amplification System Perkin Elmer # NEP116E001EA
- HRP Conjugation Kit - Lightning-Link Abcam # ab102890
- SureBlue Reserve TMB 1-Component Microwell Peroxidase Substrate, KPL Cat No. 53-00-03
- Deionized (DI) Water. Can use lab DI water.
- Purified recombinant capture and detection antibodies:

Ab	Binding domain*	Species	Isotype	Description
DH1219	NP	Human	IgG1	NP capture
HRP-DH1218	NP	Human	IgG1	NP detection
DH1041	Spike	Human	IgG1	Spike capture
HRP-DH1046	Spike	Human	IgG1	Spike detection

**SARS-CoV-2 Spike (S) and nucleocapsid/nucleoprotein (NP)*

Materials and Equipment

2
- Costar 3700 high binding 384 well plates
- Plate Washer: Biotek 406
- Plate Reader: Molecular Devices 384plus
- Corning® 500mL Vacuum Filter/Storage Bottle System, 0.22µm Filter Membrane, Product No. 430769
- Stir bar and stir plate.
- Clean sterile 1L bottles, glass or plastic
- Various plastic tubes capable of holding ranges 4 to 50ml of liquid
- Pipette tips for matching pipettes.
- Plastic wrap
- Benchtop centrifuge capable of 10,000g
- Manual single channel pipette set ranging from 2ul to 1000ul
- 12 channel high and low volume manual multichannel pipettes
- Matrix electronic 12 channel spreadable and 16 channel fixed width electronic pipettes capable of 2-125ul volumes.
- Disposable 96 well dilution plates with 300ul/well capacity

***Substitutions may be acceptable, but should be validated prior to use*

Preparation of Storable Solutions

3 **0.1 M Sodium Bicarbonate (Coating buffer)**

1. Measure 8.4g of Sodium Bicarbonate. Transfer to a 1L bottle.
2. Bring the volume up to 1 L using DI water. Mix solution until dissolved.

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3. Filter the 0.1M Sodium Bicarbonate solution using Corning® 500mL Vacuum Filter/Storage Bottle System with 0.22µm Filter Membrane in order to remove any undissolved particles.
4. Transfer the solution into new bottle, and store at room temperature. Discard after one month.

1%BSA/0.1%Tween-20 in PBS (1%BSA/T)

1. 10 g of BSA. Transfer to 1L bottle.
2. Add 40 mLs of 25X PBS and 1.0 mL of Tween 20. Bring volume to 1L with DI water. Stir the solution until dissolved. Store at 4°C. This has a shelf life of two weeks. If any turbidity occurs, discard and remake.

Assay Wash (10L)

1. In 10L bottle, add 10 mL of Tween20 and 400 mL of 25X PBS. Bring the volume up to 10L using DI water. Stir completely. This will yield in 1X PBS/0.1%Tween-20 solution.

Acid Stopping Solution (1X HCl Solution)

1. To make 1L of 1X HCl (.33 N) solution using recommended HCl, add approximately 700 mL of DI water into 1L bottle.
2. Add 27 mL of Hydrochloric Acid. Bring the volume to 1L with DI water. Mix the solution.

Assay Procedure

4 Dilute each capture antibody, DH1041 (S) and DH1219 (NP), to a final concentration of 4ug/ml in coating buffer at a volume sufficient to fill all assay wells of a 384 well plate with 15ul (see plate map at the end). Unused portions of the plate can be left empty if desired.

5 Coat assay wells of high-binding 384 well ELISA plate with 15 µL/well of coating solution. Tap plate(s) lightly to ensure all liquid settles at the bottom of the wells. Cover tightly or wrap the plate(s) with plastic wrap then incubate overnight at 4°.

6 Wash plate(s) twice with 100µL of assay wash per well. Make sure wells are empty (pat plates on paper towel if necessary).

7 Block wells with 80µL casein/well. Tap plates lightly to ensure all liquid settles at the bottom of the wells. Incubate for at least 2 hours at room temperature or overnight at 4oC. If storing overnight, wrap the plate(s) with plastic wrap.

8 Purified Recombinant Antigen Standard curve:

- In separate tubes dilute the recombinant S and NP proteins to 1ug/ml in 1%BAS/T.
- In a 96 well dilution plate perform a serial dilution in three-fold dilution ratio. Have the first well start at 0.01ug/ml (10,000pg/pm) for each protein.
- This requires a 1 to 100 dilution from each individual protein tube (at 1ug/ml) into the same starting well. Example: In the first well add 265ul 1%BAS/T. Then add 2.5ul of each 1ug/ml tube of S and NP.
- Into all other wells add 180ul of 1%BAS/T. Then transfer 90ul from the first well into the next well, serially through all 12 wells, with proper mixing.

9 Vortex or invert the test samples to mix, then centrifuge 10,000 g x 5 minutes to settle any solids.

10 After blocking the plate(s), wash one time with assay wash. Make sure wells are empty. All further steps are conducted at room temperature.

11 Following the plate layout add 10ul/well undiluted sample in triplicate to the assay plate.

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12 Add standard curve to the assay plate at a volume of 10ul/well in dulpicate.

13 To blank rows add 1%BSA/T for subtraction later.

14 Tap plate to make sure all samples are at the well bottom. Cover and incubate for 60 minutes.

15 During incubation, prepare the HRP labeled detection antibody cocktail by diluting each HRP labeled antibody to its optimal concentration in the same tube 1%BSA/T. Currently DH1046 (anti-S) is used at 0.25ug/ml and DH1218 (anti-NP) is used at 0.1ug/ml.

- The HRP labeled antibodies must be pre-labeled following the procedure outlined in the following kit: HRP Conjugation Kit - Lightning-Link Abcam #ab102890.
- The working concentration of each HRP labeled antibody must be determined by titration in a prior optimization assay as outlined briefly below:

1. Using all reagents outlined above, coat plate with the capture antibodies as specified. Then wash and block as above.
2. Prepare recombinant antigen standards by diluting each antigen separately to a final concentration of 1,000pg/ml. Add each antigen solution separately to the assay plate as outlined above.
3. Prepare a dilution of each HRP detection antibody separately, beginning at 1ug/ml serially diluting for 12 wells.
4. Incubate the HRP-detection antibody for 60 min and continue the detection as outlined in this protocol.
5. Choose a working concentration of each HRP detection antibody that is both at saturation and practical, i.e >=0.1ug/ml.

16 During incubation calculate and prepare the ELAST ELISA enhancer reagents according to the protocol provided in the kit specified in the reagents section.

- BT Solution.: Volume required = 10ul/well x # of wells + 10%. Example: If 4ml is needed (2ml DiH2O + 2ml BT diluent + 40ul BT reagent)
- HRP Solution: Volume required = 10ul/well x # or wells + 10%.Dilute HRP 1 to 500 in 1%BST/T. Example: If 4ml is needed (1%BSA/T + 8ul HRP reagent).Protect from light until use.

17 Following incubation with HRP detection antibodies, wash plate(s) twice. Make sure wells are empty.

18 To all wells add 10µL/well of BT solution. Incubate plate for 20 minutes.

19 Wash plate(s) four times.

20 Add 10µL of HRP solution. Incubate plate for 20 minutes.

21 Wash plate six times.

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22 Add 20ul/well of TMB substrate. Incubate 12 minutes.

23 Stop reaction with addition of 20ul acid stop solution. Tap plates to distribute stop solution.

24 Read plates at 450 nm between 5 to 30 minutes after stopping the reaction.

Determination of Results

25
- Average all background wells.
- Subtract background average from all wells.
- Average each set of triplicates, and the standard curve dulpicates.
- Any sample greater than OD 0.15 after subtraction is considered positive.
- The standard curve should be positive to at least 40pg/ml.

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