RESEARCH USE ONLY

Product Code: KVN
English

QuantiSARSCoV-2 Nucleocapsid Protein IgG Antibody CELISA

INTRODUCTION

SARS-CoV-2 is a novel beta-coronavirus which emerged in late 2019, causing a severe pneumonia-like illness called Covid-19. To date, the resulting pandemic has infected over 228 million people and caused more than 4.6 million deaths over 24 months. Prior to its discovery, only 6 other human coronaviruses had previously been identified, four causing a mild respiratory illness (common cold) while the other two, SARS and MERS respectively, are far more virulent, often leading to fatal respiratory disease.

As with other coronaviruses, SARS-CoV-2 seroconversion generally occurs some 7-10 days after symptoms develop, though there are reports of specific IgM and IgA detection as early as day 4. IgG antibodies appear slightly later than the other two isotypes, reaching a maximum titre from day 21 onwards, and persisting for several months after infection. In contrast, both serum IgA and IgM responses are fairly short-lived. SARS-CoV-2 has a single-stranded, positive sense 30kb RNA genome, which codes for 26 proteins. Four structural proteins, membrane (M), nucleocapsid protein (N), envelope (E) and spike protein (S), offer possible targets for immunodiagnosis and of these, the S and N are commonly used for diagnostics.

The Cellabs QuantiSARS-CoV2 Nucleocapsid Protein IgG Antibody CELISA is based on the classical indirect ELISA principle. Plates coated with highly purified recombinant N antigen are exposed to serum, plasma or dried blood sample (DBS) from patients recovering from COVID-19 infection. If specific antibodies are present, they bind to the antigen and can be subsequently measured using highly specific anti-human monoclonal antibody conjugate.

INTENDED USE

For the detection of IgG antibodies to the nucleocapsid protein of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in plasma, serum or dried blood spot (DBS) samples. Not to be used for the detection of antibodies produced from vaccinations against SARS-CoV-2. For professional use only.

CONTENTS OF THE KIT

		KVN-1plate Kit	KVN-3 plate Kit
VNMW	N antigen coated microwell plate (single use)	1 x 96-well plate	3x 96-well plates
CONTROL -	Negative Control	1 x 0.03 mL	1 x 0.09 mL
VNPT	PBS/Tween (20x)	1 x 60 mL	1 x 125 mL
VNSC	Substrate Chromogen (TMB) (20x)	1 x 0.7mL	1 x 2.5 mL
VNSB	Substrate Buffer	1 x 15 mL	1 x 50 mL
VNSS	Stopping Solution	1 x 7 mL	1 x 20 mL
CONTROL +	Positive Control IgG	1 x 0.03 mL	1 x 0.1 mL
VNPO-G	Enzyme Conjugate IgG (200x)	1 x 0.08 mL	1 x 0.24 mL

Kit Components Sold Separately

CONTROL +	Positive Control IgA (Research Use Only)	1 x 0.03 mL
CONTROL +	Positive Control IgM (Research Use Only)	1 x 0.03 mL
VNPO-A	Enzyme Conjugate IgA (200x) (Research Use Only)	1 x 0.08 mL
VNPO-M	Enzyme Conjugate IgM (200x) (Research Use Only)	1 x 0.08 mL

This kit is optimised for use with the detection of N IgG, IgM or IgA antibodies (IgM and IgA are for research use only and can be ordered separately). Store kit and components at 2-8^{o}C. Expiry dates are clearly marked on each kit component and on the box. Expiry dates do not change once opened.

MATERIALS REQUIRED BUT NOT PROVIDED

Distilled water, micropipettes and tips, clean glassware or plastic containers for solutions, humid chamber, ELISA washer, ELISA plate reader to read absorbances at a single wavelength of 450nm or at dual wavelength of 450nm and 620nm or automated ELISA systems.

PRECAUTIONS

For in-vitro diagnostic use only. Do not use after the expiry date shown on the label. If protective packaging is damaged, contact your local distributor and ask for a replacement. Treat all clinical serum or plasma samples as if they are biohazardous, use utmost precaution by using personal protective clothing and equipment and/or follow established protocols in respective laboratory for testing biohazardous materials. Inactivate samples by heat-treating to 60^{o}C for 20 minutes. Do not mix reagents form different batch numbers. Thiomerosal preservative added to some components is a poison. Exercise caution when handling these components. The stopping solution is corrosive. Avoid contact with skin, eye and mucous membranes. Ensure all dilution containers are clean before use. Avoid exposure of the substrate to light. Treat all clinical and control material as though potentially infectious and dispose of in accordance with local operating regulations. For further information, please refer to the Safety Data Sheet.

INSTRUCTIONS FOR USE

Preparation of Wash Buffer

If crystals are present, pre-warm the concentrate and stir on a magnetic stirrer until all crystals are dissolved. Alternatively, leave the 20x Wash Buffer bottle on the laboratory bench overnight to avoid crystallization, this is stable at room temperature. The working solution 1x Wash Buffer for washing step can be pre-prepared in bulk by diluting 50mL of concentration VNPT into 950mL of distilled water. Label the bottle 1x WASH BUFFER and store at 2-8^{o}C, for use up to one week.

Preparation of samples

Exercise caution when testing serum, plasma and fingerprick dried blood spot (DBS) samples. Treat all samples as biohazardous. As a precaution, inactivate potential viral particles in samples by heat-treating to 60^{o}C for 20 minutes. Samples can be heat-treated before diluting to 1:200 in working dilution PBS/T before testing (DBS to be eluted prior to heat-treatment). For quantification, construct the standard curve by serially diluting two-fold the positive control (standard) starting at a dilution of 1:100. A minimum of 8 dilutions will be required to construct the standard curve.

Dried Blood Spot samples using the Tropbio blood collection filter paper discs (Cellabs Cat. No.: FP) shall be prepared by eluting the dried blood. Elute by placing one protrusion or 'ear' into a 2mL flip-top microtube and adding 200uL of PBS/T (1x). Gently mix to submerge the "ear" and leave standing at room temperature for a minimum of 3 hours or overnight (keep at 2-8^{o}C). Ensure that the tube is thoroughly mixed before testing in the ELISA. Each 'ear' contains approximately 10uL of blood. We assume the extraction of 4uL of serum each 'ear' therefore the addition of 200uL of PBS/T is equivalent to serum/plasma dilution of 1:50. Eluted 1:50 samples are to be further diluted using 1x PBS/T to give an end dilution of 1:200 before testing.

Assay Procedure for Manual Method

1. Bring all reagents to room temperature (18-25 °C) before use.
2. Prepare test samples and Wash Buffer working dilution (1x) WASH BUFFER by following the Preparation steps outlined earlier.
3. Load 100uL each of diluted test sample and Negative Control CONTROL - sample (1:200 dilution) into designated wells.
4. Incubate for 1 hour at 37°C in a humid chamber (plastic box with lid, place a layer of wet tissues at bottom).
5. Wash the wells four times using 1x WASH BUFFER
6. Prepare working conjugate by diluting the 200x concentrate VNPO into 1x WASH BUFFER. For each 8-well strip, allow 1000uL. Dilute 5uL into 995uL of 1x WASH BUFFER
7. Load 100uL of diluted conjugate into each well.
8. Incubate for 30 minutes at 37°C in a humid chamber
9. Wash the wells four times using 1x WASH BUFFER.
10. Prepare the 1x working strength TMB VNSC by diluting concentrated Substrate Chromogen TMB VNSC in Substrate Buffer VNSB. For each 8-well strip, allow 1000uL. Dilute 50uL concentrate into 950uL Substrate Buffer VNSB.
11. Load 100uL of diluted TMB substrate into each well.
12. Incubate at Room Temperature, in the dark for 10 minutes. Development of blue colour shall be seen on positive control wells.
13. Stop the reaction by adding 50uL each well of Stopping Solution VNSS.
14. Read results on a plate reader at dual wavelength 450/620nm or 450nm single wavelength.

Assay Procedure for Automated Systems

1. Bring all reagents to room temperature (18-25 °C) before use.
2. Prepare 1x WASH BUFFER (see Preparation of Wash Buffer above) and 1x working strength conjugate. For each plate, allow 12mL of 1x conjugate (mix 0.06mL of concentrated 200x CONJUGATE in 11.94 mL of CONJUGATE DILUENT.
3. Prepare samples by diluting in 1x PBS/T at 1:200 before testing.
4. Remove required number of plates. For partly used plates, replace unused microwell strips in snap-lock foil bag and seal firmly. Store at 2-8°C.
5. For automated systems, initiate machine to prepare for the assay. The automated machine shall be configured as follows:

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6. Into the designated control wells, add 100uL of positive and negative controls in duplicates. Include duplicate blank wells using 1x wash buffer.
7. Add 100uL each of sample into designated wells.
8. Incubate the plates in a humidity chamber for 1 hour at 37°C.
9. Wash the wells four times using pre-diluted 1x working wash buffer (PBS/T).
10. Add 100µL of pre-diluted working strength CONJUGATE to each well.
11. Incubate for 30 minutes in a humid chamber at 37°C.
12. Wash the wells four times using pre-diluted 1x working wash buffer (PBS/T).
13. Prepare the 1x working strength Substrate Chromogen TMB VNSC. Allow 12.0mL for each plate (mix 0.6mL of concentrated Substrate Chromogen TMB VNSC in 11.4mL Substrate Buffer VNSB
14. Add 100uL of diluted TMB substrate into each well and incubate in the dark at room temperature for 10 minutes.
15. Add 50µL of Stopping Solution VNSS. Gently shake to mix.
16. Read the results preferably at 450nm/620nm (or 450nm).

READING AND INTERPRETATION OF RESULTS AND DIAGNOSIS

Visually

Observe the colour intensity of the control and specimen wells. The Positive Control should be blue before and yellow after stopping.

Photometrically

Read the microwell plate at 450nm or 450/620nm in a compatible ELISA plate reader, blanked against air. For the test results to be accepted the controls must read as follows:

Figure 1: Kit Performance Specifications for each valid test run.

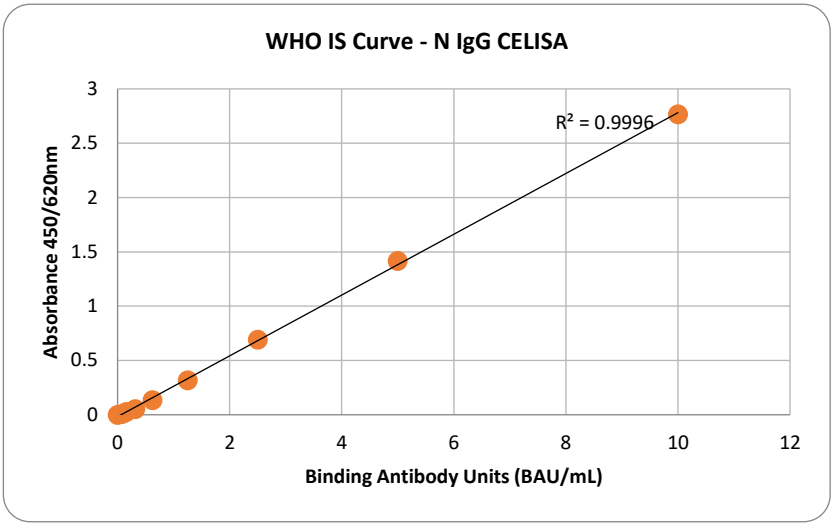
	O.D. Value (450nm or 450/620nm)
Negative Control	O.D < 0.150
Positive Control	O.D > 2.0
Blank	O.D < 0.150

Quantification using the WHO International Standard Binding Antibody Units (BAU/mL)

The First WHO International Standard antibody for SARS-CoV-2 (WHO IS) was used to calibrate the QuantiSARSCoV-2 Positive Control (Standard). To quantify Binding Antibody Units per millilitre (BAU/mL), use the Standard provided with the kit as a component. The standard is serially diluted two-fold from 1:100 (10ug/mL) to a minimum of 8 dilutions to construct a linear standard curve. The ELISA OD results at 450/620nm dual wavelength or 450nm single wavelength is blanked using the negative control. Blanked values are used to construct a linear curve against which unknown test samples can be quantified. A simple standard linear curve can be generated using a spreadsheet program eg. Microsoft Excel. Quantification using the WHO IS can only be achieved if the Standard OD values are within range and linear as shown in Figure 2. As a guide, the Standard at 10 ug/mL (1:100 dilution) must be within the blanked range OD reading of 2.8 (+/- 0.3).

Figure 2: Expected Standard Curve using the WHO IS or QuantiSARSCoV-2 N IgG Antibody CELISA calibrated Positive Control

International Unit (IU/mL)	Blanked 450/620nm OD
10	2.805
5	1.456
2.5	0.731
1.25	0.357
0.625	0.175
0.313	0.093
0.156	0.064
0.078	0.048
0.039	0.037
0.02	0.026



ASSAY PERFORMANCE

Table 1: Sensitivity and Specificity based on validation studies in Table 2.

SENSITIVITY	**92.9%**
SPECIFICITY	**99.7%**
PPV	**92.2%**
NPV	**99.8%**

***To be updated after additional evaluation results**

Validation Summary

Table 2: Validation studies using a cut-off-value (COV) of 50 BAU/mL

Samples			Positive 1:200 Dilution
Origin	Total		
Manila PNRC (MA) Box 2 pre COVID-19	92	618	Negatives 612/618
Manila PNRC (MA) Box 3 pre COVID-19	92		
Manila PNRC (A) Box 1 pre-COVID-19	191		
Aust Red Cross (ARC) preCOVID-19	92		
Papua New Guinea Students (PNGS) Pre COV	92		
NEN Pre-COVID-19	59		
NIBSC Verification Panel 14 Negatives	14		
PSG Positive samples	42	72	Positives 69/72
Positive Control (Melb)	1		
WHO References: 163, 150, 148, 144, 140 Positives	5		
WHO Reference 142	1		
NIBSC Verification Panel 23 Positives,	23		

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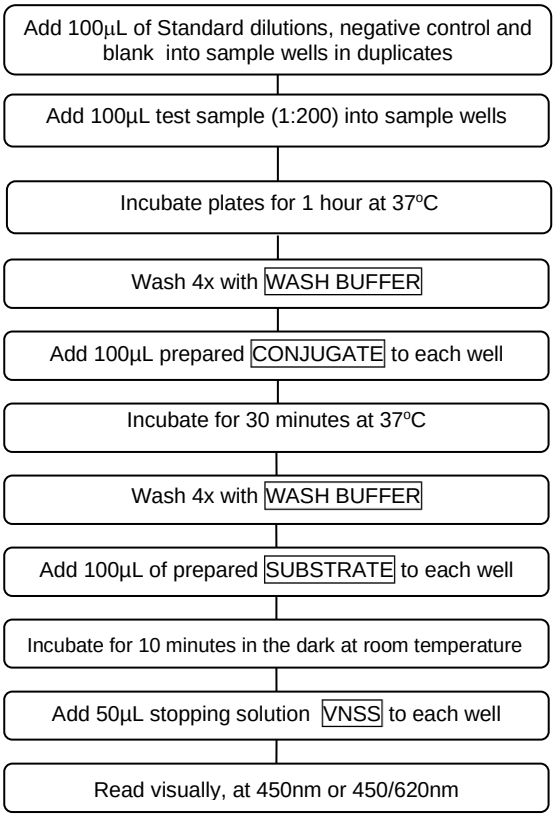
WASTE DISPOSAL
Dispose of any unused component as bio-hazardous waste. For more information, please refer to the MSDS.

INDEMNITY NOTICE
Modifications or changes made in the recommended procedure may affect the stated or implied claims and performance of the kit. Cellabs and its agents or distributors are not liable for damages under these circumstances.

FIGURE 3: QuantiSARS-CoV2 N IgG ANTIBODY CELISA Method Summary



EXPLANATION OF SYMBOLS

	Consult Instructions for Use
IVD	*In Vitro* Diagnostic Medical Device
2°C 8°C	Temperature Limitation
LOT	Batch
CONTROL +	Control Positive
CONTROL –	Control Negative
	Use By/Expiration Date
	Do Not Re-use

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