Product Manual

SARS-CoV-2 Nucleocapsid IgG ELISA Kit (RUO)

Catalog #: ENZ-KIT193-0001

1 x 96 well assay

For the latest product information, including support documentation, visit us online:
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Please read entire booklet before proceeding with the assay.

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INTRODUCTION

The SARS-CoV-2 Nucleocapsid IgG ELISA Kit (ENZ-KIT193) is designed for measuring the amount of SARS-CoV-2 nucleocapsid IgG in serum and plasma samples.

The SARS-CoV-2 virus, now referred to as COVID-19, is a single-stranded RNA coronavirus that causes respiratory infections. This novel virus is responsible for the 2020 global COVID-19 pandemic[1]. Novel virus SARS-CoV-2 belongs to the same viral family as SARS and MERS. Thus, researchers predict that the antibody generation will be similar to that of other SARS and MERS viruses. In turn, the antibodies produced against SARS-CoV-2 will be indicative of SARS-CoV-2 current or past infection[2]. Once an individual has been infected, there is an initial immune response in which IgM antibodies are produced followed by IgG antibodies. Both IgM's and IgG's are produced in the blood in response to antigen's contained on the SARS-CoV-2 virus. IgM antibodies against SARS-CoV-2 suggests a recent infection of SARS-CoV-2 has taken place, while IgG antibodies against SARS-CoV-2 suggests that the virus exposure happened in the past[2]. High levels of IgG antibodies against nucleocapsid protein of SARS-CoV-2 have been detected in sera from SARS patients. The nucleocapsid protein is an antigen for the T-cell response in a vaccine setting, inducing SARS-specific T-cell proliferation and cytotoxic activity[3]. Having a better understanding of the antibody behavior post-virus exposure will allow researchers to gain not only a better understanding of SARS-CoV-2 immune response following infection, but will also allow researchers the tools needed to figure out a way to enhance immunity to this highly infectious virus[4].

The SARS-CoV-2 virus contains several proteins including the spike glycoprotein (S), Hemagglutinin esterase (HE), small envelope (E), membrane (M), and the nucleocapsid (NC). The spike protein is a transmembrane glycoprotein that contains an RBD domain recognizing angiotensin converting enzyme (ACE2). Recognition of ACE2 allows the protein to gain entry into cells[4]. The nucleocapsid protein is a highly conserved protein and is useful because of its strong immunogenicity[5]. The nucleocapsid protein is also more stable than the spike protein with 90% amino acid homology to SARS-CoV and is less susceptible to mutations over time[3]. Nucleocapsid proteins of many coronaviruses are known to be abundantly expressed during infection. Due to its highly conserved nature and abundance during infection, the nucleocapsid protein of SARS-CoV-2 can potentially allow for better comparative data between patient samples for clinicians or between experiments for researchers. Enzo Life Sciences has developed a SARS-CoV-2 Nucleocapsid IgG ELISA that could not only be used qualitatively

for clinical sample characterization, but also semi-quantitatively for researchers who desire to study production of IgGs post-infection, discover potential vaccines and therapies, or gain understanding of the virus production time course[2,3,4].

PRINCIPLE

1. The IgG calibrators and diluted serum samples are added to the wells of a microplate coated with a recombinant SARS-CoV-2 nucleocapsid protein.
2. After the primary incubation, the wells are washed removing any unbound antibodies.
3. A horseradish peroxidase (HRP) labeled goat anti-human IgG antibody is added to each well.
4. After the secondary incubation, the wells are washed removing any unbound conjugate.
5. TMB Substrate solution is then added. The substrate generates a blue color when catalyzed by the HRP.
6. After the substrate incubation, Stop Solution is added to stop the reaction.
7. The resulting yellow color is read at 450 nm. The amount of signal is directly proportional to the level of human IgG in the sample.
8. Using the values obtained from the calibrators, the amount of IgG in the diluted sample can be determined by interpolation of a four-PL curve fitting software.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use reagents beyond the expiration date of the kit.

1. **SARS-CoV-2 Nucleocapsid Coated Microplate**
   Component No. 80-2988
   A plate using break-apart strips coated with recombinant nucleocapsid protein.

2. **SARS-CoV-2 Nucleocapsid IgG Calibrator Pack**
   Component No. 80-2993
   - **Calibrators 1-4 –** Lyophilized, Component No. 80-2983, 80-2984, 80-2985, 80-2986
     One vial each, containing 200, 50, 10 and 0ng nucleocapsid IgG

3. **SARS-CoV-2 Nucleocapsid Conjugate, 10mL**
   Component No. 80-2989
   A solution of goat anti-human conjugated horseradish peroxidase.

4. **SARS-CoV-2 Nucleocapsid Sample Diluent, 100 mL**
   Component No. 80-2987
   Tris buffered saline containing proteins and detergents.

5. **Wash Buffer Concentrate (20X), 30 mL**
   Component No. 80-1286
   One bottle containing 20x tris buffered saline with detergent.

Protect TMB substrate from prolonged exposure to light.

6. **TMB Substrate, 10 mL**
   Component No. 80-0350
   A solution of 3,3'5,5' tetramethylbenzidine (TMB) and hydrogen peroxide.

Stop solution is caustic. Use caution and keep tightly capped.

7. **Stop Solution 2, 10 mL**
   Component No. 80-0377
   A 1N solution of hydrochloric acid.

8. **SARS-CoV-2 Nucleocapsid Assay Layout Sheet, 1 each**
   Component No. 30-0355

This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at -20°C in the sealed bag provided. The wells should be used in the frame

STORAGE

Upon receipt, store entire kit at -20°C. When stored at -20°C, all components are stable until the kit's expiration date. All components in the kit are stable for 1 month at 4°C or for 2 days at room temperature. Once the lyophilized calibrators are reconstituted in sample diluent they are stable for 1 month at 4°C or 2 days at room temperature. Avoid multiple freeze thaws of the reconstituted calibrators. All components **must** be equilibrated to room temperature prior to use.

OTHER MATERIALS NEEDED

1. Deionized or distilled water.
2. Precision pipets for volumes between 1 µL and 1,000 µL.
3. Repeater pipet for dispensing 100 µL.
4. Disposable beakers for dilution buffer concentration.
5. Benchtop centrifuge.
6. Adsorbent paper for blotting.
7. Microplate reader capable of reading at an optical density of 450nm.
8. Software for extrapolating sample values from optical density reading utilized a four parameter logistic curve fit.

WARNINGS AND PRECAUTIONS

1. Observe good laboratory practices. All biological materials should be treated as potentially infectious and handled as such. They should be disposed of in accordance with established biohazardous safety procedures.
2. The SARS-CoV-2 Nucleocapsid IgG ELISA Kit workflow should be performed by qualified and trained staff to avoid the risk of erroneous results.
3. Kit components should be stored at the proper temperatures as indicated on the labels and in the storage section of the insert.
4. Due to the short kinetics of this assay, be sure that all components are brought to room temperature before use. Temperature difference can cause inter-assay variability.
5. Close attention should be paid to the timing of the assay. Small differences in the addition of the reagents to the wells can negatively affect the results.
6. Always check the expiration date prior to use of reagents. Do not use expired reagents. Do not mix components from different kit lots.
7. Always wear proper PPE, which includes a suitable lab coat, disposable gloves, an N95 or similar mask, and protective goggles.
8. Some of the kit components contain hazardous substances. Consult the appropriate material safety data sheets (MSDS) for more information.

Samples must be stored at or below -20°C to avoid loss of bioactive analyte. Avoid repeated freeze/ thaw cycles.

If buffers other than those provided are used in the assay, the end-user must determine the appropriate dilution and assay validation.

SAMPLE HANDLING

The SARS-CoV-2 Nucleocapsid IgG ELISA kit is suitable for measuring Nucleocapsid IgG levels in human serum and plasma. When diluted sufficiently into sample diluent the concentration of Nucleocapsid IgG in a sample can be determined by interpolation off the provided calibrators. Since this kit is detecting human antibodies, which are present at high levels in serum and plasma, the samples need to be diluted to a background level to remove the effect of endogenous IgG. This minimum required dilution was determined to be 1:200 (please refer to the Sample Matrix Properties section for detailed data).

Any dilution less than 1:200 may result in a falsely high signal, due to endogenous IgG. The 1:200 dilution may not be optimal for all samples as the levels of the levels of IgG could vary between sample groups. Therefore, it is up to each end user to optimize the

dilution for their unique set of samples. **Note: The actual dilution used should be included when interpolating values of the calibrator curve.**

REAGENT PREPARATION

1. **1X Wash Buffer**
   Prepare wash buffer by diluting 30 mL of the supplied Wash Buffer Concentrate with 570 mL of deionized water. The diluted wash buffer can be stored at room temperature for up to 6 months.

2. **Nucleocapsid IgG Calibrators**
   The Nucleocapsid IgG Calibrators lyophilized. Reconstitute each calibrator in 1mL of Sample Diluent to bring them to their working 1X concentration to prior to using in assay. Vortex well. Any unused 1x calibrators can be stored at 4°C for up to 1 month or room temperature for up to 2 days. Avoid multiple freeze thaws.

ASSAY PROTOCOL

Bring all reagents to room temperature for at least 1 hour prior to use.

Refer to the Assay Layout Sheet to determine the number of strips to be used. Remove unneeded strips and return them, with the desiccant, to the plate bag. Store the unused wells at -20°C.

1. To minimize variability, all reagents must be equilibrated to room temperature prior to running the assay. Alternatively, the entire kit can be placed at room temperature the night before the assay needs to be run.
2. Dilute all samples 1:200 in Sample Diluent.
3. Add 100µL of each of the four Nucleocapsid IgG Calibrators to the appropriate wells. Leave the Blank wells empty.
4. Add 100µL of diluted samples to appropriate wells.
5. Incubate the plate at room temperature for 10 minutes.
6. Empty the contents of the wells and wash by adding a full well volume (~350uL) of 1x Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
7. Add 100µL of Nucleocapsid Conjugate to each well, except for the Blank wells.
8. Incubate the plate at room temperature for 10 minutes.

For best results it is recommended that calibrators and samples should be run in duplicate.

9. Empty the contents of the wells and wash by adding a full well volume (~350uL) of 1x Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 2 more times for a total of 3 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
10. Add 100µL TMB Substrate to all wells, including the Blank, and incubate at room temperature for 10 minutes.
11. Add 100µL Stop Solution 2 to complete the reaction.

    Note: It is important to add the stop solution to the wells of the plate in the same order as the TMB substrate was applied.
12. Read the plate at an optical density of 450nm.

SAMPLE MATRIX PROPERTIES

Minimum Required Diluted (MRD)

Human serum, EDTA plasma, and heparin plasma were serially diluted in sample diluent to determine at what dilution sample levels were below background. The MRD was determined to be 1:200.

	Average OD (450nm)			IgG (ng/mL)		
Dilution	Serum	EDTA Plasma	Heparin Plasma	Serum	EDTA Plasma	Heparin Plasma
1	2.547	2.438	2.018	144.459	133.413	97.485
6.25	0.611	0.552	0.421	21.399	19.005	13.861
12.5	0.376	0.347	0.273	12.092	10.970	8.132
25	0.230	0.214	0.180	6.467	5.844	4.490
50	0.147	0.145	0.127	3.195	3.114	2.380
100	0.107	0.1085	0.097	1.542	1.606	1.109
200	0.092	0.09	0.090	0.865	0.797	0.540
400	0.079	0.0735	0.075	0.281	0.129	0.281

Sample Linearity

Human serum, EDTA plasma, heparin plasma and sample diluent were diluted 1:200 in sample diluent. Each matrix was then spiked with recombinant nucleocapsid IgG and further serially diluted 1:2 in sample matrix. Linearity at each dilution is expressed as a percentage.

Dilution	Serum	EDTA Plasma	Heparin Plasma	Sample Diluent
400	94%	83%	79%	83%
800	104%	96%	92%	91%
1600	112%	97%	100%	96%
3200	119%	107%	109%	102%
6400	123%	107%	111%	106%
12800	100%	100%	100%	100%

Sample Linearity (with known positive samples)

Positive CoV-2 human neat serum and plasma samples were serially diluted 1:2 into sample diluent. Sample values were interpolated off the calibrator curve and linearity of each sample was assessed between 200 and 2.5ng/mL.

Dilution	Serum #1	Serum #2	Serum #3	Serum #4	Serum #5	EDTA Plasma #1	EDTA Plasma #2	EDTA Plasma #3	Heparin Plasma #1	Heparin Plasma #2	Heparin Plasma #3
20	OOR	OOR	OOR	100%	OOR	OOR	100%	100%	100%	OOR	100%
40	120%	OOR	87%	113%	OOR	OOR	98%	101%	100%	OOR	127%
80	107%	OOR	162%	106%	OOR	121%	116%	109%	111%	OOR	121%
160	106%	OOR	76%	109%	OOR	108%	115%	99%	104%	OOR	131%
320	106%	89%	75%	110%	121%	104%	114%	110%	OOR	126%	116%
640	104%	160%	80%	108%	114%	110%	116%	109%	OOR	107%	104%
1280	125%	99%	OOR	114%	102%	104%	92%	112%	OOR	116%	104%
2560	78%	109%	OOR	OOR	109%	100%	OOR	OOR	OOR	107%	103%
*ng/mL at 1:200 dilution	6,060	28,440	17,600	4020	27,260	11,420	2,780	2740	4,700	39,420	3,780

* Interpolated value was multiplied by 200.

Spike and Recovery

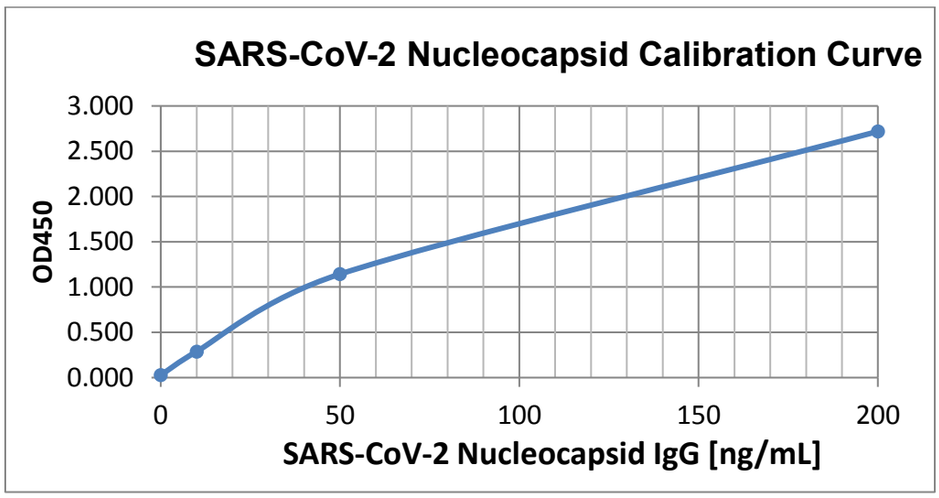
Sample matrices were diluted 1:100 (2X the desired final MRD) in sample diluent. An equivalent amount of each calibrator was added to each so that the final spike concentrations were 100, 25 and 5ng/mL at 1:200 of each matrix. Recovery is represented as the percentage of actual IgG concentration divided by expected recovery.

	% Recovery		
	100ng/mL Spike	25ng/mL Spike	5ng/mL Spike
Serum	89.4%	81.3%	83.0%
EDTA Plasma	91.2%	85.0%	92.3%
Heparin Plasma	88.9%	90.9%	106.6%

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Sample	SARS-CoV-2 Nucleocapsid IgG [ng/mL]	Mean OD	Net OD
Calibrator 1	200	2.76	2.72
Calibrator 2	50	1.18	1.14
Calibrator 3	10	0.33	0.29
Calibrator 4	0	0.07	0.03



CALCULATION OF RESULTS

After using the plate reader to determine the optical density values of each well at 450nm, several options are available for the calculation of SARS-CoV-2 Nucleocapsid IgG in samples. We recommend that the data be calculated semi-quantitatively by interpolation off the calibrator curve using an immunoassay software package utilizing a 4-parameter logistic curve fitting program (4-PL). The results can also be analyzed using only the OD values. **Note: For the purpose of determining whether a sample is considered positive or negative for SARS-CoV-2 IgG, the initial 1:200 dilution of sample needs to be taken into account. To do this multiply the interpolated value by 200. This is on top of any other dilution that is performed.**

1. **Semi-quantitative calculation**
   Input the data into software that contains 4-PL curve fitting and interpolate the sample values of the calibrator curve with the units being ng/mL.

   Interpretation of Results
   Negative ≤ 2000 ng/mL
   Implies no SARS-CoV-2 IgG is present.
   Positive > 2000 ng/mL
   Implies SARS-CoV-2 IgG is present.

2. **Interpretation via OD values**
   Compare the OD values of the calibrators to the OD values of the samples.

   Interpretation of Results
   Negative = $OD_{sample\ x}$ dilution factor ≤ 200 x $OD_{Calibrator\ 3}$
   Implies no SARS-CoV-2 IgG is present.
   Positive = $OD_{sample\ x}$ dilution factor > 200 x $OD_{Calibrator\ 3}$
   Implies SARS-CoV-2 IgG is present.

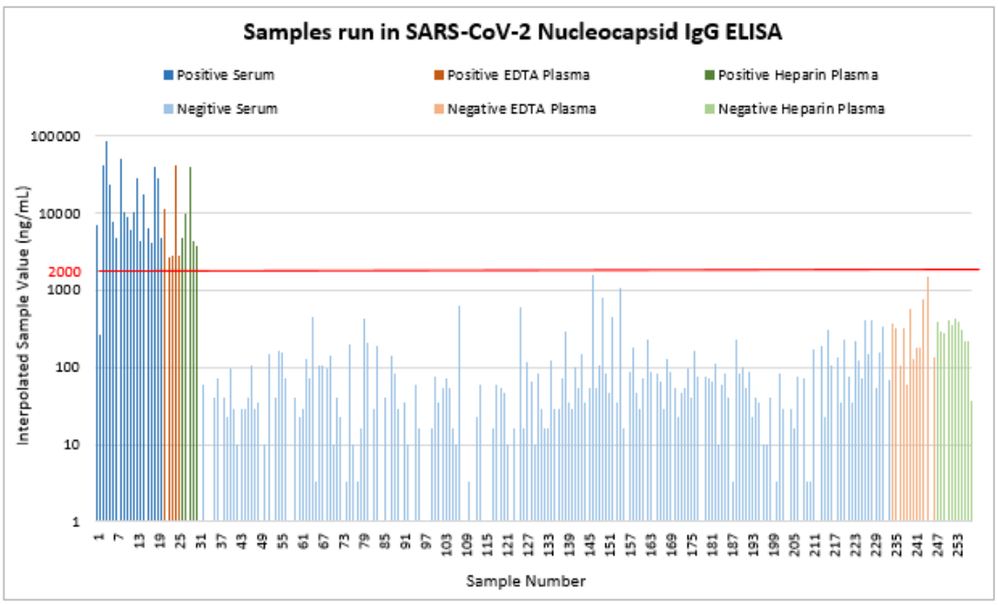
QUALITY CONTROL

Calibrators must always be included on each plate to determine the validity of any test results. Each test should be considered an implied result as to the presence of SARS-CoV-2 Nucleocapsid antibodies. It is recommended that the samples be repeated in this assay or rerun in a separate assay, to confirm the result.

PERFORMANCE CHARACTERISTICS

Cutoff Value Determination

The cutoff value to distinguish known positive vs negative samples was assessed by running negative samples collected prior to September 2019 (n=227) and positive samples (n=30) confirmed by both RT-PCR and IgG ELISA. For each sample, the interpolated value was multiplied by the dilution performed (1:200) to obtain the final sample value. The highest negative value obtained was 1548ng/mL. The average negative value 116ng/mL. The highest positive value obtained was 84,440ng/mL (read above the range of the calibrators). The average positive value was 16,420ng/mL. Of the 30 positive samples, there was 1 false negative that read 252ng/mL (Sample #2). Based on the data the cutoff value for a sample to be considered positive was determined to be >2000ng/mL.



Sensitivity and Specificity

The SARS-CoV-2 Nucleocapsid IgG ELISA Kit was assessed in a population group of 257 specimens (combination of male and female). The specimens were collected from outside vendors and Enzo Clinical Labs, Inc, Farmingdale, NY, USA. Negative samples (n=227) were obtained prior to September 2019. Positive samples (n=30) were confirmed by both RT-PCR and in a separate serological IgG ELISA of SARS-CoV-2.

			Negative	Positive	Sensitivity	Specificity
Enzo SARS Cov-2 Nucleocapsid IgG ELISA	Serum	Positive (> 2000ng/mL)	0	19	100%	95%
		Negative (< 2000ng/mL)	203	1		
	EDTA Plasma	Positive (> 2000ng/mL)	0	5	100%	100%
		Negative (< 2000ng/mL)	13	0		
	Heparin Plasma	Positive (> 2000ng/mL)	0	5	100%	100%
		Negative (< 2000ng/mL)	11	0		

Cross Reactivity

SARS-CoV-2 nucleocapsid IgG used to make the calibrators exhibited zero cross reactivity against SARS-CoV-2 ECD, SARS-CoV-2 spike S1, and SARS-CoV-2 RBD domains via both western blot and antigen down ELISA.

Interfering Substances

The following concentration of potentially interfering substances were spiked into plasma or serum containing SARS-CoV-2 Nucleocapsid IgG. The returned values are expressed as a percentage of the expected value for each matrix.

Interferent	Concentration	Plasma	Serum
None	0 mg/mL	95%	102%
Albumin (60mg/mL)	60 mg/mL	92%	98%
Bilirubin (0.2mg/mL)	0.2 mg/mL	90%	97%
Cholesterol (3mg/mL)	3 mg/mL	90%	94%
Thyroglobulin (3mg/mL)	3 mg/mL	88%	97%
Hemoglobin (1mg/mL)	1 mg/mL	95%	101%
Lipemic Sample	High	92%	92%
Hemolyzed Sample	High	104%	103%

Intra-assay Precision

Intra-assay reproducibility expressed in terms of both concentration and OD values of 18 replicates of each provided calibrator.

Control	Conc. (ng/mL)	SD	%CV
Control 1	179.78	7.82	4.35
Control 2	83.18	3.14	3.79
Control 3	35.52	1.91	5.38

Inter-assay Precision

Inter-assay reproducibility expressed in terms of both concentration and OD values of 18 replicates of each provided calibrator.

Control	Conc.(ng/mL)	SD	%CV
Control 1	155.77	9.92	6.37
Control 2	72.93	5.62	7.71
Control 3	34.02	1.49	4.37

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NOTES

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