



LEGEND MAX™

ELISA Kit



SARS-CoV-2 Nucleocapsid Human IgG

Cat. No. 448107

ELISA Kit for Accurate Quantitation of
SARS-CoV-2 Nucleocapsid Human IgG
from Serum and Plasma

BioLegend, Inc.
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

For Research Purposes Only. Not for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of BioLegend is strictly prohibited.



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Introduction:

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the infectious agent which is the cause of the global pandemic of Coronavirus disease 2019 (COVID19). The SARS-CoV-2 virion contains many proteins including Spike (S), nucleocapsid (N), envelope (E), and membrane (M). The Nucleocapsid protein of human coronavirus is expressed at high level in infected cells because it has a critical role in stabilizing the viral RNA, and the assembly of the virion. Many studies showed that anti-Nucleocapsid antibody can be detectable in the blood of COVID19 patients more than 160 days after disease onset. In addition to indicating past exposure to SARS-CoV-2, quantification of anti-Nucleocapsid antibody can also be used as a parameter for assessing the severity of the COVID19 disease.

The LEGEND MAX™ SARS-CoV-2 Nucleocapsid Human IgG ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with the SARS-CoV-2 Nucleocapsid protein. The detection antibody is a biotinylated anti-Human IgG, Fc fragment specific, monoclonal antibody. This kit is specifically designed for the accurate quantitation of anti-Nucleocapsid IgG, from serum and plasma. It is analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity	Volume	Part #
SARS-CoV-2 Nucleocapsid Human IgG Pre-Coated 96-well Strip Micro-plate	1 plate		750002355
SARS-CoV-2 Nucleocapsid Human IgG Detection Abs	1 bottle	12 mL	750002357
SARS-CoV-2 Nucleocapsid Human IgG Lyoph. Std.	1 vial	lyophilized	750002360
Avidin HRP	1 bottle	12 mL	77897
Assay Buffer B	1 bottle	25 mL	79128
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

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Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Polypropylene tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Standard	The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.
Avidin-HRP	Store opened reagent bottles at 2° - 8°C and use within 1 month
Assay Buffer B	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents,

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handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at $< -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at $1,000 \times g$. Remove serum layer and assay immediately or store serum samples at $< -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at $1,000 \times g$ within 30 minutes of collection. Assay immediately or store plasma samples at $< -70^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

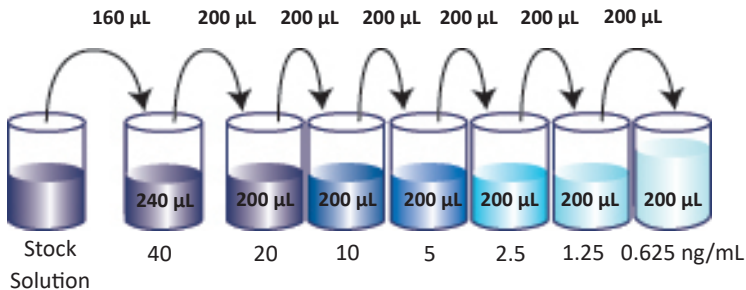
Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized SARS-CoV-2 Nucleocapsid Human IgG Lyoph. Std. by adding the volume of Assay Buffer B to make the 100 ng/mL standard stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general, a 1000-folds dilution in Assay Buffer B is recommended for serum, EDTA Plasma, and Citrate Plasma samples. A minimum 2000-folds dilution is recommended for Heparin Plasma samples. However, samples can be diluted further to fit within the range of the assay as determined by the end user.

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 400 μL of the 40 ng/mL top standard by adding 160 μL of the 100 ng/mL standard stock solution into 240 μL Assay Buffer B. Perform six two-fold serial dilutions of the 40 ng/mL top standard in separate tubes using Assay Buffer B as the diluent. Thus, the SARS-CoV-2 Nucleocapsid Human IgG standard concentrations in the tubes are 40 ng/mL, 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL and 0.625 ng/mL, respectively. Assay Buffer B serves as the zero standard (0 ng/mL).



4. Wash the plate 4 times with at least 300 μL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 μL of Assay Buffer B to each well that will contain either standard dilutions or samples. Then add 50 μL of standard dilutions or samples to the appropriate wells.
6. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 2 hours at room temperature with no shaking.
7. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
8. Add 100 μL of SARS-CoV-2 Nucleocapsid Human IgG Detection Abs solution to each well, seal the plate and incubate at room temperature for 1 hour

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with no shaking.

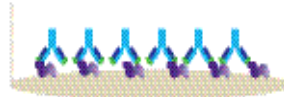
9. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
10. Add 100 μ L of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes with no shaking.
11. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
12. Add 100 μ L of Substrate Solution F to each well and incubate for 20 minutes in the dark. Wells containing SARS-CoV-2 Nucleocapsid Human IgG should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
13. Stop the reaction by adding 100 μ L of Stop Solution to each well. The solution color should change from blue to yellow.
14. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

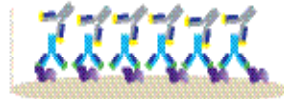
1. Wash 4 times.
Add 50 μ L Assay Buffer B to standard wells and sample wells



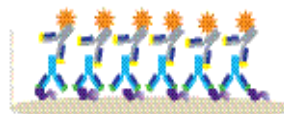
2. Add 50 μ L of standard or sample, incubate 2 hr, RT, no shaking



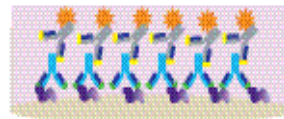
3. Wash 4 times
Add 100 μ L of SARS-CoV-2 Nucleocapsid Human IgG Detection Abs solution. Incubate 1 hr, RT, no shaking



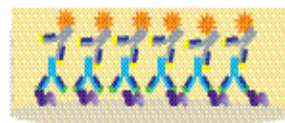
4. Wash 4 times
Add 100 μ L Avidin-HRP solution
Incubate 30 min, RT, no shaking



5. Wash 5 times
Add 100 μ L Substrate Solution F
Incubate 20 min, RT, in the dark



6. Add 100 μ L Stop Solution



7. Read absorbance at 450 nm and 570 nm

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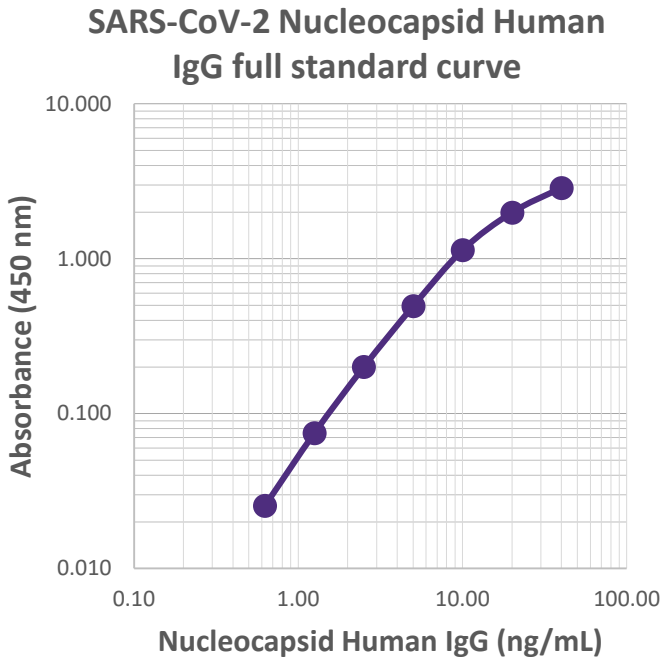
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with the IgG concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown IgG concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the IgG concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



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Performance Characteristics:

Specificity: This kit recognizes natural and recombinant SARS-CoV-2 Nucleocapsid Human IgG. No cross reactivity was observed when this kit was used to analyze the following antibodies at 400 ng/mL.

Human anti-SARS-CoV-2 S Protein S1 antibody	clone AM009105 (BioLegend Catalog # 938501) clone AM002414 (BioLegend Catalog # 938501) clone AM038105 (BioLegend Catalog # 940701) clone AM006415 (BioLegend Catalog # 938601)
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Sensitivity: The minimum detectable concentration of SARS-CoV-2 Nucleocapsid Human IgG 0.091 ± 0.021 ng/mL (n=6).

Recovery: SARS-CoV-2 Nucleocapsid Human IgG at 3 different concentrations was spiked into at least 3 different human samples each of Serum, Citrate Plasma, EDTA Plasma, and Heparin Plasma. Sample recovery was then analyzed with the LEGEND MAX™ SARS-CoV-2 Nucleocapsid Human IgG ELISA Kit.

Sample Type	N	% Recovery
Serum	4	89%
Citrate Plasma	3	92%
EDTA Plasma	3	85%
Heparin Plasma	3	85%

Spike and Linearity: Natural human samples were first diluted to 1000 folds, spiked with 20 ng/mL of SARS-CoV-2 Nucleocapsid hIgG, and then diluted 2 fold in serial to produce samples within the dynamic range of the kit. Samples were then assayed to determine the dilutional linearity.

Sample Type	N	% Linearity
Serum	4	106%
Citrate Plasma	3	96%
EDTA Plasma	4	101%
Heparin Plasma	3	105%

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Intra-Assay Precision: Two samples containing different SARS-CoV-2 Nucleocapsid Human IgG concentrations were tested on one plate with 12 replicates.

Concentration	Sample 1	Sample 2
Number of Replicates	12	12
Mean Concentration (ng/mL)	11.6	3.0
Standard Deviation	0.7	0.1
%CV	6%	4%

Inter-Assay Precision: Two samples containing different SARS-CoV-2 Nucleocapsid Human IgG concentrations were tested in ten independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	10	10
Mean Concentration (ng/mL)	12.3	2.8
Standard Deviation	1.3	0.3
%CV	11%	9%

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Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN ₃)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

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Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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