# ReSARS™ CoV-2 (N) IgG ELISA

## (Human anti-SARS-CoV-2 N-specific IgG Antibody)

### For *In Vitro* Diagnostic Use Only

**For Prescription use only. Use of this test is limited to laboratories certified to perform high complexity testing.**

# INSTRUCTIONS FOR USE

## INTENDED USE

**This test has not been reviewed by the FDA**.

The ReSARS™ CoV-2 (N) IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the semi-quantitative detection of N-specific IgG antibodies to SARS-CoV-2 in human serum or plasma (K+-EDTA, Li+-heparin, Na+-citrate). The ReSARS™ CoV-2 (N) IgG ELISA is intended for use as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection. At this time, it is unknown for how long antibodies persist following infection and if the presence of antibodies confers protective immunity. The ReSARS™ CoV-2 (N) IgG ELISA should not be used to diagnose or exclude acute SARS-CoV-2 infection. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, to perform high complexity tests.

Results are for the detection of SARS-CoV-2 antibodies. IgG antibody to SARS-CoV-2 is generally detectable in blood several days after initial infection, although the duration of time antibodies are present post-infection is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

The sensitivity of SARS-CoV-2 IgG early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.

Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

The product is not to be used to test donated blood.

## SUMMARY AND EXPLANATION OF THE TEST

In December 2019, a novel coronavirus emerged in Wuhan City, Hubei Province, China. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the etiologic agent for coronavirus infectious disease 2019 (COVID-19) which causes acute respiratory disease and is often fatal in cases requiring ventilator-assisted breathing support. New features of the SARS-CoV-2 genome make it highly contagious and contributed to COVID-19 rapid spread which has become a global pandemic.

The COVID-19 pandemic has created a world-wide clinical, public health and economic crisis. Initial responses have addressed the challenges of diagnosis and treatment, reconfiguring and supplying health systems and implementing public health strategies for contact tracing, social distancing and border controls to suppress transmission[1,2]. Population-wide surveillance will be critical in addressing major gaps in knowledge about risk factors for infection, transmission dynamics[3,4], seasonal and geographic patterns, correlations between immune markers and reinfection, and long-term immunologic, physiologic and clinical sequelae of infection including mortality[5,6].

Zalgen Labs, LLC developed a nucleocapsid (N) protein-based ELISA for the semi-quantitative measurement of human IgG levels in response to infection with COVID-19. Viral nucleocapsids are highly immunogenic in humans, with humoral immunity often superseding the response against other viral antigens, temporally and in magnitude. The ReSARS™ CoV-2 (N) IgG ELISA measures the levels of circulating N protein-specific human IgG in the plasma or serum of subjects who have been infected with COVID-19 and who have initiated immunoglobulin G seroconversion. The assay detects IgG bound to recombinant SARS CoV-2 N immobilized in microwells, returning a semi-quantitative value corresponding to level of

antigen-specific IgG in a test plasma or serum sample. The assay contains positive and negative controls and an IgG reference standard for generation of a standard curve. The assay is a medium complexity ELISA requiring preparation of working buffers from provided stock solutions and direct use of optimized conjugate, substrate, and stop solutions. Operation of a plate washer and an ELISA plate reader are required, according to manufacturer's instructions.

## PRINCIPLES OF THE PROCEDURE

This test is a direct-format ELISA detecting human IgG antibody specific for SARS-CoV-2 N protein. Diluted samples, Reference, Positive Control and Negative Control are incubated in microwells coated with recombinant SARS-CoV-2 N protein. Incubation allows the anti-SARS-CoV-2 antibody present in the human diagnostic samples to react with the immobilized N protein. After the removal of unbound serum or plasma proteins by washing, Goat anti-human IgG labeled with horseradish peroxidase (HRP), is added forming complexes with the bound anti-SARS-CoV-2 IgG antibody. Following another washing step, the bound enzyme-antibody conjugate is assayed by the addition of TMB substrate. Color develops in the wells at an intensity proportional to the concentration of anti-SARS-CoV-2 IgG antibody in the sample.

Optical Density (O.D.) results are obtained by reading the absorbance (A450nm minus A620nm) using an ELISA plate reader. It is recommended that the user establish a cut-off for the study population using SARS-CoV-2 sero-negative samples. It is also recommended that IgG positive convalescent COVID-19 samples from the study population be included in each test as an additional reference sample.

## REAGENTS

Store at 2–8°C. Do Not Freeze.
Each ReSARS™ CoV-2 (N) IgG ELISA kit contains the following reagents:

| Component | 2-plate kit | 10-plate kit |
|---|---|---|
| ReSARS CoV-2 Nucleocapsid Protein Coated Microwell (resealable bag with desiccant) | Two 12x8 plates | Ten 12x8 plates |
| ReSARS CoV-2 Anti-Hu IgG ELISA HRP Conjugate Solution (Blue) | 1 x 30 mL bottle | 1 x 120 mL bottle |
| ELISA Sample Diluent 2 | 1 x 120 mL bottle | 3 x 250 mL bottles |
| Negative Control (human plasma, lyophilized) | 1 x 0.25 mL vial | 5 x 0.25 mL vial |
| Anti-N IgG Positive Control (human plasma, liquid) | 1 x 0.1 mL vial | 5 x 0.1 mL vials |
| Anti-N IgG Reference (human plasma, liquid) | 1 x 0.1 mL vial | 5 x 0.1 mL vials |
| ELISA One-Component Substrate (TMB and $H_2O_2$); ready to use (Amber Bottle) | 1 x 30 mL bottle | 1 x 120 mL bottle |
| ELISA Stop Solution (2% methanesulfonic acid) (Red Cap) | 1 x 30 mL bottle | 1 x 120 mL bottle |
| ELISA Wash Concentrate (33X PBS/Tween 20) | 1 x 120 mL bottle | 2 x 120 mL bottles |

## WARNINGS AND PRECAUTIONS

### For *In Vitro* Diagnostic Use Only. Not for use in diagnostic procedures.

**SARS-CoV-2 virus (the etiological agent of COVID-19) is classified as NIAID Category C agent. Handling of infectious blood, serum, or nasal/oral swabs may be done in biocontainment 2 (BSL-2) facilities. Use of standard precautions is highly recommended including face shields, masks or N95 respirators, disposable gowning and gloves. Decontamination equipment and solutions should be readily available. Biohazardous wastes should be autoclaved and/or incinerated.**

1. Human source material used to prepare the controls included in this kit has been tested. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material (Universal Precautions).
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.

4. One-component substrate can cause irritation to the eyes and skin. Absorption through the skin is possible. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.
5. Certain components are labeled with the following: Irritating to eyes (R 36). Avoid contact with skin and eyes (S 24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). If swallowed, seek medical advice immediately and show container or label (S 46).

   Irritant  . Biological Risk  .

## SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Serum or plasma are the preferred sample matrices. Blood should be collected by venipuncture, and the serum separated from the cells by centrifugation after clot formation. If not tested immediately, specimens should be stored at 2–8°C. If specimens are to be stored for more than 72 hours, they should be frozen at –20°C or below. Avoid repeated freezing and thawing. Do not use grossly hemolyzed, icteric, or lipemic serum as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If plasma is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation following the blood tube manufacturer's recommendations. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable in order to minimize platelet contamination. If not tested immediately, plasma samples should be stored as described for serum.

Performance of directly testing sputum, nasopharyngeal swabs, or oropharyngeal swabs has not been evaluated. The product is not to be used to test donated blood. Viral transport media may require additional dilution in the Sample Diluent provided.

## PROCEDURE

### MATERIALS PROVIDED:

ReSARS™ CoV-2 (N) IgG ELISA kit; see "Reagents," for a complete listing.

### MATERIALS REQUIRED BUT NOT PROVIDED:

- Reagent grade water to prepare Wash Solution and reconstitute lyophilized References and Controls
- Graduated cylinders
- Precision pipettors capable of delivering between 10 µL and 1000 µL, with appropriate tips
- Miscellaneous glassware appropriate for small volume handling
- Flask or bottle, 1 liter
- Disposable gloves
- Plate reading spectrophotometer capable of reading absorbance at 450nm (with a reference within 620nm to 650nm if available)
- Multichannel pipettors capable of delivering to 8 or 12 wells simultaneously

## PROCEDURAL NOTES

1. Bring samples and kit reagents to ambient temperature (18–30°C) and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. The plate reader should be programmed for blank subtraction.
3. Good washing technique is critical for optimal performance of the assay. An automated microtiter plate washing system should be used with bleach added to the waste reservoir.
4. IMPORTANT: Failure to adequately remove residual Wash Solution can cause inconsistent color development of the Substrate Solution.
5. Use a multichannel pipettor capable of delivering to 8 or 12 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.

6. Carefully controlled timing of all steps is critical. All controls and samples must be added within a five-minute period.
7. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
9. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
10. Incubation temperatures above or below ambient temperature (18–30°C) when required may contribute to inaccurate results.
11. Avoid contamination of reagents when opening and removing aliquots from the primary vials.
12. Do not use kit components beyond expiration date.
13. Do not use components from different kit lot numbers.

## REAGENT PREPARATION

**Wash Solution**: Measure 30 mL of Wash Concentrate (33X) and dilute to 1 liter with reagent grade water. The pH of the final solution should be 7.35 ± 0.1. Store unused Wash Solution in the refrigerator at 2–8°C . Discard if the solution shows signs of microbial contamination.

**Lyophilized Negative Control:** Reconstitute Negative Control with 0.250mL reagent grade water. Mix gently for several minutes until completely dissolved. Unused portion should be store at 2-8°C for up to 7 days or stored frozen (-20°C or less) for longer periods.

## ASSAY PROCEDURE

1. Remove any microwell strips that will not be used from the frame and store them in the bag provided.
2. Prepare a five-point Reference curve: Label five tubes for Reference 1 – 5.

   In tube #1, prepare a 1:101 dilution of Reference in Sample Diluent by adding 10 µL Reference to 1000 µL Sample Diluent.

   Add 750 µL of Sample Diluent to tubes # 2-5.

   Remove 250µL from dilution tube #1, transfer to dilution tube #2 and mix well.

   Repeat this 4-fold serial dilution series through tube 5.

   The value of the Reference is indicated on the vial label. The value of Reference dilutions 2 - 5 are calculated by dividing Reference value by each Reference dilution factor (DF).

   Example:

| Dilution # | DF | Volume to Add | | Sample Diluent Volume | | Reference Value |
|---|---|---|---|---|---|---|
| 1 | - | 10 uL Reference | + | 1000 uL | = | 100.0 units/mL |
| 2 | 4 | 250 uL Dilution #1 | + | 750 uL | = | 25.0 units/mL |
| 3 | 16 | 250 uL Dilution #2 | + | 750 uL | = | 6.25 units/mL |
| 4 | 64 | 250 uL Dilution #3 | + | 750 uL | = | 1.56 units/mL |
| 5 | 256 | 250 uL Dilution #4 | + | 750 uL | = | 0.39 units/mL |

3. A reagent blank control must be run in duplicate on each plate. This well is then treated the same as sample wells in subsequent assay steps.
4. Duplicate determinations are recommended. Prepare a 1:101 dilution of the normal control and samples in Sample Diluent; e.g., 10µL sample added to 1000µL Sample Diluent equals a 1:101 sample dilution.
5. Add 100µL of prepared Reference, Negative Control, Positive Control, diluted samples, and reagent blank to the appropriate microwells.
6. Incubate 30 minutes at ambient temperature (18-30°C).

7. After the incubation is complete, wash 4 x 300µL/well with 1X wash solution. (For automated washing use overflow wash steps).
8. Add 100µL anti-human IgG HRP-conjugated antibody solution to all the wells containing Reference curve, human samples, negative control, and reagent blank.
9. Incubate for 30 minutes at ambient temperature (18-30°C).
10. After the incubation is complete, wash 4 x 300µL/well with 1X wash solution as in step 7. (For automated washing use overflow wash steps)
11. Add 100µL One-Component Substrate to each well and incubate for **10 minutes** at ambient temperature (18-30°C) while protected from light. Blue color will develop in wells with positive samples.
12. Add 100µL Stopping Solution (2% methanesulfonic acid) to each well to stop the enzyme reaction. Blue substrate will turn yellow and colorless substrate will remain colorless. Blank or zero the plate reader against the duplicate Reagent Blank. Read the OD of each well at 450 nm (650 nm reference, if available). The OD values should be measured within 5 minutes after the addition of Stopping Solution.

## QUALITY CONTROL

1. Reagent Blank maximum OD ≤ 0.075. Readings greater than OD 0.075 may indicate possible reagent contamination or inadequate plate washing.
2. Reference curve minimum OD (minus Reagent Blank) of 1:100 dilution ≥1.500, minimum OD (- Blank) of 1:6,400 dilution > 0.150.
3. Negative Control maximum OD (minus Reagent Blank) ≤ 0.100. Estimated units/mL from Reference curve within label range.
4. Positive Control (3-fold dilution above cut-off) minimum OD (minus Reagent Blank) >0.250. Estimated units/mL from Reference curve within label range.
5. OD values for duplicates of the controls or patient samples should be within 25% CV of the mean OD value, for samples with OD readings greater than 0.250.

## RESULTS

### Calculation

1. Calculate the mean OD values for the duplicates of the Reference dilutions, Positive Control, Negative Control, Reagent Blank, and samples.
2. Subtract mean OD A650nm reference from mean OD A450nm.
3. Subtract resulting mean OD of Reagent Blank from all reference dilutions, controls, and samples.
4. Estimate the concentration of IgG by plotting the mean O.D. obtained for each dilution of IgG Reference on the Y-axis against the corresponding IgG Reference (unit/mL) value on the X-axis using curve fitting software. A 4-Parameter curve fit calculation is recommended.
5. Ensure that all quality control parameters have been met (see Quality Control) before reporting results.

### Interpretation of Results

- The Negative Cut-Off for each lot of ReSARS™ CoV-2 (NP) IgG ELISA is included in the Certificate of Analysis.
- Samples with an estimate IgG concentration (units/mL) above the Negative Cut-off are SARS-CoV-2 NP-specific IgG positive.
- Samples with an estimate IgG concentration (units/mL) below the Negative Cut-off are SARS-CoV-2 NP-specific IgG negative.
- Samples with ODs higher than the first Reference dilution (1:100) may cause a greater than (>) error using curve fitting software. These samples would be considered positive but further dilution of sample and retesting may be considered.

- Samples with negative ODs after Reagent Blank subtraction may cause a less than (<) error using curve fitting software. The samples would be considered negative but retesting may be considered.
- Samples with estimated IgG concentration at or near Negative cut-off may be considered for retesting or testing of follow-up sample if patient is suspected of IgG seroconversion.

## LIMITATIONS OF THE TEST – FOR *IN VITRO* DIAGNOSTIC USE ONLY

Anti-SARS-CoV-2 IgG antibody levels obtained with this assay are not for use in diagnostic procedures.

Cross-reactivity with non-SARS-CoV-2 coronaviruses has not been evaluated.

The presence of Rheumatoid Factor (RF) in COVID-19 samples may interfere with ELISA methods by binding to antibodies. The presence of RF should be considered when evaluating results.

Testing COVID-19 samples containing excess hemoglobin, lipids, and/or bilirubin is not recommended as these substances may interfere with the results of the assay.

## PERFORMANCE CHARACTERISTICS

### ReSARS CoV-2 (N) IgG ELISA Kit – Clinical Performance Estimates

Positive IgG Cut-off ≥ 2 units/mL

| | | *SARS-CoV-2 qRT-PCR* | |
|---|---|---|---|
| | | ***qPCR Positive*** | ***Negative/Normal*** |
| ***ReSARS CoV-2 (N) IgG ELISA*** | ***Positive*** | ***61*** | ***1*** |
| | ***Negative*** | ***1*** | ***119*** |

*Positive Percent Agreement = 98.4% (61/62; 95$^{th}$% CI 91.3 – 100%)*

*Negative Percent Agreement = 99.2% (119/120; 95$^{th}$% CI 95.4 – 100%)*

*Positive Predictive Value = 98.4% (61/62; 95$^{th}$% CI 91.3 – 100%)*

*Negative Predictive Value = 99.2% (119/120; 95$^{th}$% CI 95.4 – 100%)*

*Accuracy = 98.9% (180/182; 95$^{th}$% CI 96.1 – 99.9%)*

*Dx Likelihood Ratio = 118.1*

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## SYMBOL LEGEND

| | | | | | | | |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  |  |  |
| Manufacturer | Batch Code | Use by/ Expiry Date | Temperature Limitations | Irritant | Biological Risk | Catalog Number | Consult Instructions for Use (Package Insert) |

## WARRANTY

Zalgen Labs, LLC disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Zalgen Labs, LLC be liable for consequential damage.

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