SARS-CoV-2 N-Protein Salivary IgG ELISA Kit

For Research Use Only
Not for use in Diagnostic Procedures

Item No. 1-1260, (Single) 96-Well Kit;
1-1260-5, (5-Pack) 480 Wells

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Page | 1

TABLE OF CONTENTS

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Page | 2

Intended Use

The Salimetrics® SARS-CoV-2 N-Protein Salivary IgG ELISA kit is an enzyme-linked immunoassay specifically designed and validated for the qualitative measurement of human IgG specific to the SARS-CoV-2 Nucleocapsid protein (N-protein) in oral fluid.

It is intended for surveillance testing and not for diagnostic use. This assay kit was optimized and validated for performance in human oral fluid and has not been validated for other human sample types, such as human serum or plasma.

Surveillance testing for antibodies to the SARS-CoV-2 virus can be used to determine if an individual may have been exposed to or infected with this virus, and also can be used to understand the percentage of people within a population or community that have developed antibodies to the virus (known as "surveillance tests," or sero-surveys). (From the FDA guidance document (https://www.fda.gov/media/137599/download))

- When used for surveillance, the results can help determine how widely the virus has spread in communities and how far the pandemic has progressed. Results from tests used for surveillance only are generally not shared with individual patients and are critical for understanding the extent of and risk factors associated with infection.
- Testing individuals may help identify who has developed antibodies against SARS-CoV-2. The results of ongoing research are needed before it is known whether these antibodies are associated with protection from future infection. Current results can help inform who may qualify to donate blood that can be used to manufacture convalescent plasma.

Please read the complete kit insert before performing this assay. Failure to follow kit procedure and recommendations for saliva collection and sample handling may result in unreliable values.

For further information about this kit, its application, or the procedures in this insert, please contact the technical service team at Salimetrics or your local sales representative.

Introduction

The novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was initially identified in the Winter of 2019 with an outbreak designation soon after, at the end of the year (1, 2). The virus rapidly spread achieving global pandemic status in March 2020, and resulted in widespread containment and confinement public health measures in most countries throughout the world.

In addition to virus exposure, there is an urgent need to better understand the levels and duration of protective immunity for public health considerations. The Salimetrics SARS-CoV-2 N-Protein Salivary IgG ELISA assay meets the technical requirement for high sensitivity and specificity performance, while using a saliva sample, allowing for central testing at scale. The main source of salivary IgG antibodies is serum, so it is not surprising that salivary IgG antibodies directly reflect the specificity and activity of those found in serum (3, 4). Oral fluid is, thus, an easily accessible surrogate to serum or plasma in this regard and enables salivary serology studies, surveillance tests or sero-surveys (5). Testing can help determine who has developed antibodies against SARS-CoV-2. Importantly, we have determined that human IgG withstands conditions commonly used for viral heat inactivation (60° or 65°C for 30 min and 95°C for 5 min). The main utility of antibody tests for SARS-CoV-2 supported by the WHO, CDC, FDA and AMA are surveillance studies and the Salimetrics N-Protein IgG ELISA kit meets this need.

Antibody levels decrease in the serum and saliva of COVID-19 patients over time (6-8), and importantly vary depending on disease severity (9, 10). In fact a higher number of asymptomatic participants become seronegative at 60 days indicating a true decline over a 2-month period rather than as an artifact of assay performance (6, 7). Therefore, our assay is benchmarked on validated commercial serum kit performance instead of molecular tests. Maximizing the likelihood of antibody detection, N protein was chosen as an advantage since it represents the most immunodominant protein in the coronavirus family.

Test Principle

This is a salivary serological ELISA kit where viral Nucleocapsid antigen is coated on microtiter plates and human IgG antibodies in test saliva samples are detected using an Anti-Human IgG detection antibody linked to horseradish peroxidase (HRP). After each incubation, unbound components are washed away. The levels of IgG bound to the viral antigen are measured by the reaction of the HRP enzyme to the substrate tetramethylbenzidine (TMB). This reaction produces a blue color. A yellow color is formed after stopping the reaction with an acidic solution. The optical density is read on a standard plate reader at 450 nm. The total amount of anti-IgG HRP Enzyme Conjugate detected is proportional to the amount of anti-SARS-CoV-2 IgG present in the sample. Qualitative cut-off values are determined for each run based on controls provided in the kit. The cut-off OD is used to divide sample OD values to produce signal/cut-off ratio in a simple calculation with values above 1.1 considered positive and below 0.8 negative. Samples reading between these values are considered borderline and a repeat test is recommended for these samples and/or a second collection one or two weeks later and repeat test.

Safety Precautions

Read Safety Data Sheets before handling reagents.

Hazardous Ingredients

Liquid Stop Solution is caustic; use with care. We recommend the procedures listed below for all kit reagents.

Handling

Follow good laboratory safety practices when handling kit reagents. Personal protective equipment is recommended including laboratory coats, gloves, and safety goggles. Wipe up spills using appropriate absorbent materials while wearing protective clothing. Follow local regulations for disposal.

Emergency Exposure Measures

In case of contact, immediately wash skin or flush eyes with water for 15 minutes. Remove contaminated clothing. If inhaled, remove individual to fresh air. If individual experiences difficulty breathing call a physician.

The above information is believed to be accurate but is not all-inclusive. This information should be used only as a guide. Salimetrics will not be liable for accidents or damage resulting from misuse of product.

Safety Data Sheets are available by contacting Salimetrics at support@salimetrics.com (See www.salimetrics.com for alternative contact options).

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Page | 5

General Kit Use Advice

- This kit uses break-apart microtiter strips. You may run less than a full plate. Unused wells must be stored at 2-8°C in the foil pouch with desiccant and used in the frame provided.
- Avoid microbial contamination of opened reagents. Salimetrics recommends using opened reagents within one month. Store all reagents at 2-8°C.
- The quantity of reagent provided with a single kit is sufficient for three partial runs. The volume of wash buffer and enzyme conjugate prepared for assays using less than a full plate should be scaled down accordingly, keeping the same dilution ratio.
- Do not mix components from different lots of kits.
- We recommend saving all reagents until data analysis has confirmed a successful run to facilitate troubleshooting if necessary.
- Prior to sample addition, please label each strip to assure plate orientation and sample order when data is acquired on plate reader.
- To ensure highest quality assay results, pipetting of samples and reagents must be done as quickly as possible (without interruption) across the plate. Ideally, the process should be completed within 20 minutes or less.
- When using a multichannel pipette to add reagents, always follow the same sequence when adding all reagents so that the incubation time is the same for all wells.
- When running multiple plates, or multiple sets of strips, controls and blanks must be run with each individual plate and/or set of strips.
- The temperature of the laboratory may affect assays. Salimetrics kits have been validated at 68-74°F (20-23.3°C). Higher or lower temperatures may affect OD values.
- Routine calibration of pipettes and other equipment is critical for the best possible assay performance.
- When mixing plates during assay procedures, avoid speeds that spill the contents of the wells.

Storage

All unopened components of this kit are stable at 2-8°C until the kit's expiration date.

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Page | 6

Specimen Collection

Acidic or high sugar foods can compromise assay performance by lowering sample pH and influencing bacterial growth. To minimize these factors, rinse mouth thoroughly with water 10 minutes before sample is collected.

Passive Drool:
Collect whole saliva by unstimulated passive drool. Donors may tilt their head forward, after allowing the saliva to pool on the floor of the mouth, and then pass the saliva through the Saliva Collection Aid (SCA) into a polypropylene vial. Collection protocols/methods are available online at www.salimetrics.com (https://salimetrics.com/how-do-i-collect-saliva/) or upon request. The source of IgG antibodies to SARS-CoV-2 is from serum and may therefore be affected by salivary flow rate. Avoiding any conditions that would provide a diluted sample, such as stimulating salivary production or collecting large volumes (over 2 mL) should be avoided. A sample collected upon awakening in the morning is preferred as levels of total antibody are highest within 30 minutes of waking. We emphasize that testing for total IgG, using the Salimetrics total IgG ELISA kit, is recommended to qualify passive drool samples for adequate levels of total IgG (above 5 µg/mL) to confidently verify negative samples. It is possible that 10-15% of passive drool samples may have inadequate levels of IgG if a morning sample is not collected.

Record the time and date of specimen collection.

Oral mucosal transudate (OMT) or gingival crevicular fluid (GCF):
To collect an IgG enriched sample, donors may use a specific device for that function that targets collection to the gumline and produces an OMT or GCF sample that ranges well above the passive drool levels of IgG. This sample is preferable and may not require sample qualification with the Salimetrics total IgG ELISA assay.

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Page | 7

Sample Handling and Preparation

After collection, it is important to keep samples cold to avoid bacterial growth (and loss of SARS-CoV-2 IgG) in the specimen. Ambient shipping is possible if the ambient temperature is below 22°C and shipped overnight. At higher temperatures, the sample should be kept at 4-8°C until shipped to the laboratory on cold packs. In general, it is recommended to refrigerate samples within 30 minutes of collection, if possible, and freeze at or below -20°C within 24 hours of collection for longer term storage. Samples may be stored at -20°C for up to 6 months. For longer term storage, refer to the Salimetrics Collection and Handling Advice page on our website. At central sites, samples should be frozen upon arrival and stored at -20° or -80°C.

With most sample collection methods, the Salimetrics total IgG assay should be used to qualify the total IgG levels of the samples on the day of the test. This is to assure that a negative test is not simply the result of a dilute sample. Samples must have total IgG levels above 5 μg/mL to conclude if a sample is a true negative in the assay. However, samples can have high levels of anti-viral antibodies and thus be positive with less than 5 μg/mL. In this case these positives should not be excluded by the 5 μg/mL total IgG criteria.

On day of assay, thaw the saliva samples completely, vortex, and centrifuge at 1500 x g for 15 minutes. Freezing saliva samples will precipitate mucins. Centrifuging pellets mucins and other particulate matter which may interfere with antibody binding and affect results. Samples should be at room temperature before making dilutions. Handle carefully so as to not disturb the mucin pellet. Pipette clear sample into appropriate dilution tubes. It is recommended to transfer the saliva avoiding the pellet to a new tube if samples will be tested for other analytes. Re-freeze saliva samples as soon as possible after running assay. Re-centrifuge saliva samples each time that they are thawed. Avoid multiple freeze-thaw cycles.

Saliva samples must be diluted for this assay. See Procedure for details.

Materials Supplied with Single Kit

	Item	Quantity/Size
1	**SARS-CoV-2 N-Protein IgG ELISA Microtiter Break-Apart Plate** Coated with purified SARS-CoV-2 N-protein.	1/96 well
2	**Positive Control** Formulated for stability when stored at 4ºC. Use as is. Contains: buffer, preservative, no human serum products.	1 vial / 1 mL
3	**Negative Control** Formulated for stability when stored at 4ºC. Use as is. Contains: buffer, preservative, no human serum products.	1 vial / 1.5 mL
4	**Anti-Human IgG HRP Enzyme Conjugate** Contains: Mouse Anti-Human IgG antibody conjugated to HRP, preservative.	1 bottle / 12 mL
5	**SARS-CoV-2 N-Protein IgG Sample Diluent**	1 bottle / 15 mL
6	**Wash Buffer Concentrate 10X** Dilute before use according to Reagent Preparation. Contains: phosphate buffer, detergent, preservative.	1 bottle / 100 mL
7	**TMB Substrate Solution** Non-toxic, ready to use.	1 bottle / 25 mL
8	**Stop Solution**	1 bottle / 12.5 mL
9	**Adhesive Plate Covers**	2

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Page | 9

Materials Needed But Not Supplied

- Precision pipettes to deliver 100 and 150 µL,
- Precision multichannel pipette to deliver 50 µL and 100 µL
- Vortex
- Plate rotator with 0.08 - 0.17-inch orbit capable of 500 rpm
- Microplate reader with capabilities to read 450 nm and 600 to 650 nm
- Computer software for data reduction
- Reagent reservoirs
- Deionized water
- Small disposable polypropylene tubes for dilution of samples
- Pipette tips
- Centrifuge capable of 1500 x g

Reagent Preparation

- Bring all reagents to room temperature and mix before use. A minimum of 1 hour is recommended for the 15 mL of Sample Diluent to come to room temperature.
- Bring Microtiter Plate to room temperature before use. ***It is important to keep the foil pouch with the plate strips closed until warmed to room temperature, as humidity may have an effect on the coated wells.***
- Prepare 1X wash buffer by diluting Wash Buffer Concentrate (10X) 10-fold with room-temperature deionized water (100 mL of Wash Buffer Concentrate (10X) to 900 mL of deionized water). ***Dilute only enough for current day's use and discard any leftover reagent.*** (If precipitate has formed in the concentrated wash buffer, it may be heated to 40°C for 15 minutes. Cool to room temperature before use in assay).

Procedure

Step 1: Read and prepare reagents according to the Reagent Preparation section before beginning assay. Determine your plate layout, below is a suggested layout. We recommend negative controls be run in triplicate for cut-off value determination, and saliva samples be run in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Ctrl-negative										
B	Ctrl-negative	Ctrl-negative										
C	Ctrl-positive	Ctrl-positive										
D	SMP-1	SMP-1										
E	SMP-2	SMP-2										
F	SMP-3	SMP-3										
G	SMP-4	SMP-4										
H	SMP-5	SMP-5										

Step 2: Keep the desired number of strips in the strip holder and place the remaining strips back in the foil pouch. Reseal the foil pouch with unused wells and desiccant. Store at 2-8°C.

Step 3: Dilute Samples:

- Prepare a 1:2 dilution of the saliva by pipetting 150 µL of saliva into 150 µL of SARS-CoV-2 IgG Sample Diluent in a dilution plate or individual tubes (not provided). Mix well.

OR

- Add 50 µL of SARS-CoV-2 IgG Sample Diluent to each well of the test plate and 50 µL of saliva into the sample well to make a 1:2 dilution of the sample. Mix well.

Step 4:

- Pipette 100 µL of Positive Control, Negative Control and diluted saliva samples into appropriate wells.
- If running a blank well, leave this well empty.

Place adhesive cover (provided) over plate. Mix plate on a plate rotator at 500 rpm and incubate for a total of 1 hour at room temperature, while shaking continuously.

Step 5: Wash the plate 4 times with 1X wash buffer. An automated plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 6: Using a multichannel pipette, add 100 µL of SARS-CoV-2 IgG Enzyme Conjugate to each well, except the blank well.

Step 7: Place adhesive cover provided over plate. Mix plate on a plate rotator at 500 rpm and incubate for a total of 1 hour at room temperature, while shaking continuously.

Step 8: Wash the plate 4 times with 1X wash buffer. An automated plate washer is recommended. However, washing may be done by gently squirting wash buffer into each well with a squirt bottle, or by pipetting 300 µL of wash buffer into each well and then discarding the liquid over a sink. After each wash, the plate should be thoroughly blotted on paper towels before turning upright. If using a plate washer, blotting is still recommended after the last wash.

Step 9: Add 100 µL of TMB Substrate Solution to each well with a multichannel pipette.

Step 10: Incubate the plate in the dark (covered) at room temperature for 30 minutes, mixing first for 5 minutes on a plate rotator at 500 rpm.

Step 11: Add 50 µL of Stop Solution with a multichannel pipette.

Step 12:

- Mix on a plate rotator for 3 minutes at 500 rpm. If green color remains, continue mixing until green color turns to yellow. Be sure all wells have turned yellow.
  Caution: Spillage may occur if mixing speed exceeds 600 rpm.
- Wipe off bottom of plate with a water-moistened, lint-free cloth and wipe dry.
- Read in a plate reader at 450 nm. Read plate within 10 minutes of adding Stop Solution. For best results, a secondary filter correction at 600 to 650 nm is recommended.

Quality Control and Cut-Off Value Determination

The Salimetrics Positive and Negative Controls should be run with each assay. When calculating and interpreting assay results, each plate should be individually considered, irrespective of the number of concurrently run plates. Results are based on the Optical Density (OD) of each sample divided by the cut-off value (CO) calculated specifically for that plate. If the readings for the CO are based on a single filter plate reader, the results should be calculated by subtracting the OD of the Blank well from the sample and control OD value. Reference subtracted values are recommended (Reference Filter between 600-650 nm may be used for correction) and if dual filters are used, you do not require running of a Blank well. However, if dual filter plate readers are not available, single filter plate reader OD450 values are acceptable.

Quality control (QC). The assay results are valid only if the QC criteria are fulfilled. Negative and Positive Controls must be included on every plate, and their OD/CO ratios must fall within expected ranges for assay results to be considered valid.

- The OD values of the Blank well, if run, containing only TMB and Stop solution, is < 0.080 at 450 nm.
- The OD value of the Positive Control must be > 0.5.
- The OD values of the Negative Control must be < 0.1 If one of the Negative Control OD values does not meet the QC criteria, it may be discarded, and the remaining two values used in the CO calculation. If two or more Negative Control values do not meet the above-mentioned QC criteria, the test is considered invalid and must be repeated.

Cut-off value calculation (CO) = Negative Control (NC) + 0.25
(Negative Control = the mean Optical Density value for three negative controls). If the Negative Control mean is < 0.02, use 0.02 as the value.

Example:
1. Negative Control (NC) Values:
   Sample 1 = 0.0245
   Sample 2 = 0.0055
   Sample 3 = 0.011
2. Positive Control (PC) Values
   Sample 1 = 0.541
   Sample 2 = 0.615
   All samples are within acceptable range.
3. Calculation of NC for cut-off value determination =
   (0.0245 + 0.0055 + 0.011)/3 = 0.014 NC which is < 0.02 so 0.02 is used henceforth for the cut-off calculation.
4. Cut-off calculation (CO) = 0.02 + 0.25 = 0.27

Calculations

1. Compute the average optical density (OD) for all duplicate wells.
2. Calculate the signal (OD) to cut-off value (OD) ratio:

$$\frac{\text{OD of the clinical sample}}{\text{Cut-off value determined}} = \text{Ratio}$$

Ratio < 0.8:   Negative
Ratio ≥ 0.8 to < 1.1:   Borderline (retest)
Ratio≥ 1.1:   Positive

In case of Borderline results, a definitive determination is not possible. We recommend a sample should be recollected from that participant for re-testing with this assay.

A new set of controls must be run with each full or partial plate.

Example Results

The results shown below are for illustration only, using 0.25 as the cut-off value, and should not be used to calculate results from another assay.

Sample	Average OD	Signal/Cut-off	Determination
1	0.045	0.18	Negative
2	0.022	0.088	Negative
3	0.080	0.32	Negative
4	4.302	17.28	Positive
5	1.736	6.944	Positive
6	0.457	1.826	Positive

Limitations

- See "Specimen Collection" recommendations to ensure proper collection of saliva specimens and to avoid interfering substances.

- Any duplicates of samples that produce CV% above 15 or samples whose signal/cut-off is in the borderline zone (0.8-1.1) should be followed by additional testing and evaluation. Likewise, if total IgG is below 5 ug/mL and the test result is negative, then the sample should also be recollected.

Salivary Human Total SARS-CoV-2 N-Protein IgG Performance Characteristics

Precision

The intra-assay precision was determined from the mean of 20 replicates of three samples in the high, mid, and low range for OD values on a single plate.

Saliva Sample	N	Mean OD*	Standard Deviation	Coefficient of Variation (%)
1	20	2.13	0.04	2.1%
2	20	0.60	0.01	2.3%
3	20	0.10	0.01	6.9%

* Reference subtracted OD values (OD $_{450}$ minus OD $_{630}$)

The inter-assay precision was determined from assay runs of 20 replicates of three samples in the high, mid, and low range on 4 separate plates.

Saliva Sample	N	Mean OD*	Standard Deviation	Coefficient of Variation (%)
1	80	2.09	0.10	4.7%
2	80	0.60	0.02	3.7%
3	80	0.10	0.01	7.5%

* Reference subtracted OD values (OD $_{450}$ minus OD $_{630}$)

Test performance: Sensitivity and Specificity

Sensitivity

Passive drool samples were collected on the same day as serum from PCR positive confirmed patients and run on the SARS-CoV-2 Salivary N-Protein IgG ELISA assay. Serum immunoassay results were obtained using the Epitope Diagnostics Inc. N-Protein IgG assay (Cat. No. KT-1032) following manufacturer's protocol. Results from the EDI kit was compared to the Salimetrics kit using the formula above to determine the outcome of the tests. If samples were borderline they were not included in the analysis. Of the 24 samples tested, 22 reported ratios above 1.1 and were considered positive, and two samples represented False Negative results.

Specificity

To determine the sensitivity 85 pre-COVID-19 collected samples (prior to December 2019) were run on the SARS-CoV-2 N-Protein IgG ELISA assay. The mean and standard deviation was calculated and a cut-off of two standard deviations established. Sample OD was divided by this cut-off OD and the ratio used to determine positive, borderline, or negative results for each of the 85 samples. Of 85 samples tested, 2 samples had ratios above 1.1 and represented False Positive results.

Assay	Performance Measure	Estimate of Performance
N-protein IgG	Sensitivity (PPA)	92% (22/24)
N-protein IgG	Specificity (NPA)	98% (83/85)

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Seller's Limited Warranty

"Seller warrants that all goods sold hereunder will be free from defects in material and workmanship. Upon prompt notice by Buyer of any claimed defect, which notice must be sent within thirty (30) days from date such defect is first discovered and within three months from the date of shipment, Seller shall, at its option, either repair or replace the product that is proved to Seller's satisfaction to be defective. All claims should be submitted in written form. This warranty does not cover any damage due to accident, misuse, negligence, or abnormal use. Liability, in all cases, will be limited to the purchased cost of the kit.

It is expressly agreed that this limited warranty shall be in lieu of all warranties of fitness and in lieu of the warranty of merchantability. Seller shall not be liable for any incidental or consequential damages that arise out of the installation, use or operation of Seller's product or out of the breach of any express or implied warranties."

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Page | 18