Human SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit

For the qualitative determination of human novel coronavirus nucleoprotein (SARS-CoV-2 NP) IgG antibody concentration

Catalog #100-0686 1 Kit

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Product Description

The Human SARS-CoV-2 Nucleoprotein IgG Antibody ELISA Kit is designed for the qualitative detection of human SARS-CoV-2 nucleoprotein (NP) IgG antibody in biological fluids such as serum and plasma.

Coronaviruses (CoVs), including SARS-CoV-2, are enveloped and contain single-stranded (positive-sense) RNA associated with a nucleoprotein. These nucleoproteins are abundantly produced within CoV-infected cells, where their primary function is to package the viral genome into flexible, helical ribonucleoprotein complexes called nucleocapsids. The nucleocapsid binds to genomic RNA and is understood to interact with viral membrane proteins during virion assembly, therefore playing a critical role in ensuring the efficiency of viral transcription, translation, and assembly. In host cells, the nucleocapsid can directly impact normal cell functions and cause cell-cycle deregulation, increased COX-2 production, and inhibition of interferon production.

The assay is based on the indirect ELISA method, in which samples are added to ELISA strip plates pre-coated with human novel coronavirus nucleoprotein. Antibodies present in the sample specific for the coronavirus nucleoprotein will bind to the pre-coated antigen. The captured antibodies are detected by addition of an anti-human IgG-conjugated horseradish peroxidase (HRP). Addition of the chromogenic enzyme substrate 3,3',5,5' tetramethylbenzidine (TMB) results in a colored product with an intensity directly proportional to the concentration of human novel coronavirus nucleoprotein IgG antibody in the sample.

Product Information

All components listed below are stable until expiry date (EXP) on label. Kit may be shipped at room temperature (15 - 25°C) but should be stored at 2 - 8°C upon receipt. Once components are opened, store at 2 - 8°C and use within 1 month.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	DESCRIPTION
Human SARS-CoV-2 NP IgG ELISA Plate	300-0285	1 plate	Store at 2 - 8°C.	Plate (12 strips x 8 wells) coated with SARS-CoV-2 nucleoprotein (NP)
Human SARS-CoV-2 NP IgG Negative Control	300-0286	800 µL	Store at 2 - 8°C.	Simulated serum matrix with no antibody
Human SARS-CoV-2 NP IgG Positive Control	300-0287	800 µL	Store at 2 - 8°C.	Recombinant anti-SARS-CoV-2 nucleoprotein IgG antibody
Human IgG HRP-Conjugated Detection Antibody	300-0288	120 µL	Store at 2 - 8°C.	Horseradish peroxidase (HRP)-conjugated anti-human IgG antibody (100X)
Human IgG HRP Antibody Diluent	300-0289	20 mL	Store at 2 - 8°C.	For dilution of detection antibody
Adhesive Plate Covers	300-0304	4 covers	Store at 2 - 8°C.	For covering plates during incubation
Stop Solution*	300-0303	10 mL	Store at 2 - 8°C.	4 N Sulfuric acid
TMB Substrate	300-0302	10 mL	Store at 2 - 8°C.	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution
ELISA Diluent	300-0300	2 x 20 mL	Store at 2 - 8°C.	For dilution of samples
Wash Buffer (25X)	300-0301	20 mL	Store at 2 - 8°C.	Concentrated buffer solution for washing plates

*Please refer to the Safety Data Sheet (SDS) for hazard information.

Materials Required But Not Included

- Biohazard safety cabinet certified for Level II handling of biological materials
- Microplate reader set at a wavelength of 450 nm and (optional) a second correction wavelength set at 540 nm or 570 nm
- 37°C incubator
- ELISA plate washer: Automatic (adaptable for ELISA strip plates) or manual (e.g. multi-pipette or squirt bottle)
- Pipettor with appropriate tips
- Beakers, flasks, and graduated cylinders necessary for reagent preparations
- Tubes for standard and sample dilutions
- Timer
- Absorbent paper
- Deionized or distilled water

Preparation of Reagents and Materials

A. Wash Buffer

NOTE: Use clean or disposable glass or plasticware for preparation and storage of wash buffer.

NOTE: If crystals have formed in the concentrate, warm to room temperature (15 - 25°C) and mix gently until the crystals have completely dissolved.

Dilute Wash Buffer (25X) 1 in 25 with distilled or deionized water.

Example: Prepare 500 mL wash buffer (1X) by adding 20 mL Wash Buffer (25X) to 480 mL distilled or deionized water.

B. ELISA Plate

1. Allow plate to adjust to room temperature (15 - 25°C) before opening the bags.
2. Plan the experiment to include the positive control (2 wells), the negative control (2 wells), a background control (2 wells), a blank (2 wells), and test samples.
3. Assemble the required number of strips in the plate frame. Store the remaining strips in the foil bag containing the desiccant at 2 - 8°C for up to 1 month.

C. Samples

Refer to instructions below for preparing serum and plasma. Avoid using lipemic, hemolyzed, or contaminated samples, as these may yield unreliable results. Fresh samples are recommended, to reduce protein degradation and denaturation risk. However, samples to be used within 5 days can be stored at 2 - 8°C. For samples to be used within 1 month, store at -20°C; for storage for 1 - 2 months, store samples at -80°C to avoid loss of bioactivity and contamination.

NOTE: Duplicate wells for all samples are recommended. For samples with a high protein concentration, it is advisable to prepare several dilutions for testing. Remove any particulate matter by centrifugation or filtration prior to use.

Serum

1. Using a serum separator tube (SST), allow samples to clot at room temperature (15 - 25°C) for 2 hours or overnight at 2 - 8°C.
2. Centrifuge at 1000 x *g* for 15 minutes. Remove serum fraction and transfer to a fresh tube.
3. Dilute serum 1 in 100 in ELISA Diluent; this dilution may need to be optimized for individual applications. Samples containing high levels of protein will require further dilution.

   NOTE: If not used immediately, aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Plasma

1. Use EDTA, citrate or heparin as an anticoagulant when collecting plasma.
2. Within 30 minutes of collection, centrifuge plasma at 1000 x *g* for 15 minutes at 2 - 8°C. Remove and discard serum fraction, then transfer plasma to a fresh tube.
3. Dilute plasma 1 in 100 in ELISA Diluent; this dilution may need to be optimized for individual applications. Samples containing high levels of protein will require further dilution.

   NOTE: If not used immediately, aliquot and store at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

D. Detection Antibody

1. Centrifuge the vial of Human IgG HRP-Conjugated Detection Antibody prior to opening to bring all material to the bottom of the vial.
2. Dilute 1 in 100 in Human IgG HRP Antibody Diluent.

   Example: Add 10 µL of Human IgG HRP-Conjugated Detection Antibody to 990 µL of Human IgG HRP Antibody Diluent.

Directions for Use

Please read the entire protocol before proceeding.

NOTE: Cross-contamination of reagents may invalidate assay results. Permanently labeled, dedicated, multi-channel pipettor reservoirs for reagents are recommended.

NOTE: In all washing steps, each well must be thoroughly washed by filling with wash buffer, allowing to sit for 2 minutes, then blotting dry by tapping the plate upside down on absorbent paper.

NOTE: Centrifuge thawed samples to bring all material to the bottom of the vial before proceeding with the assay.

1. Bring all reagents and samples to room temperature (15 - 25°C), except the TMB Substrate, which should be kept at 2 - 8°C until use.
2. Prepare wash buffer (1X), reagents, samples, and the ELISA plate as described in Preparation of Reagents and Materials.
3. Add 100 µL/well of Human SARS-CoV-2 NP IgG Negative Control, Positive Control, or diluted serum or plasma samples to the ELISA plate. Leave the blank wells empty.

   NOTE: Duplicate wells for all samples are recommended.
4. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 30 minutes.
5. Aspirate wells. Wash each well with 5 x 200 µL of wash buffer and blot dry.
6. Add 100 µL/well of Detection Antibody. Leave blank wells empty.
7. Cover the plate with an Adhesive Plate Cover and incubate at 37°C for 30 minutes.
8. Aspirate wells. Wash each well with 5 x 200 µL of wash buffer and blot dry.
9. Add 90 µL/well of TMB Substrate to all wells (including blank wells).

   NOTE: TMB Substrate should be colorless or light blue until added to the plate. Protect TMB Substrate from light.
10. Incubate at 37°C in the dark for 20 minutes.
11. Add 50 µL/well of Stop Solution to all wells (including blank wells), in the same order as TMB Substrate addition. Gently tap the plate to ensure thorough mixing.

    NOTE: Use care when handling Stop Solution. Please refer to the Safety Data Sheet (SDS) for hazard information.

    NOTE: The color in the wells will turn from blue to yellow after addition of Stop Solution. Wells that are green indicate they are not thoroughly mixed.
12. Within 10 minutes of adding Stop Solution, measure the absorbance at 450 nm in a microplate reader. If possible, use a correction wavelength of 540 nm or 570 nm. Blank the reader using wells containing TMB Substrate and Stop Solution only.

    NOTE: Before measuring absorbance, remove any air bubbles in the wells using a small hypodermic needle or a pipette tip.
13. Determine whether the sample is positive or negative for human SARS-CoV-2 nucleoprotein IgG antibodies by comparing the sample well with the negative control well, as follows:
    - Negative: The optical density (OD) of the sample is < 2.1X the OD of the negative control
    - Positive: The optical density (OD) of the sample is ≥ 2.1X the OD of the negative control

Performance of the Assay

- Precision: The intra-assay precision of this assay is < 15% (CV). The inter-assay precision of this assay is < 15% (CV).
- Specificity: This assay has high specificity for the detection of human novel coronavirus nucleoprotein (SARS-CoV-2 N) IgG antibody. No significant cross-reactivity or interference is known to occur between human novel coronavirus nucleoprotein (SARS-CoV-2 N) IgG antibody and analogues.

Troubleshooting

PROBLEM	POSSIBLE CAUSE	RECOMMENDED ACTION
Absorbance values too low	Incubation time too short	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Undiluted Wash Buffer (25X)	Dilute Wash Buffer (25X) with distilled or deionized water as described in Preparation of Reagents and Materials, section A.
	Incorrect dilution of Detection Antibody	Follow the directions for dilution of Detection Antibody in Preparation of Reagents and Materials.
	Wash step omitted before adding Detection Antibody	Follow sequence of incubation and wash steps as outlined in Directions for Use.
Absorbance values too high	Incubation time too long	Follow the directions for the multiple incubation steps outlined in Directions for Use.
	Insufficient washing	Ensure that each well is washed 5X with 200 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps.
	Incorrect dilution of Detection Antibody	Follow the directions for dilution of Detection Antibody in Preparation of Reagents and Materials.
Low absorbance readings despite good color development in wells	Incorrect wavelength setting on the ELISA reader	Check that the measuring wavelength is set at 450 nm and that the ELISA reader has the correct filter for this wavelength.
High background	Insufficient washing	Ensure that each well is washed 5X with 200 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step.
	Incorrect dilution of Detection Antibody	Follow the directions for dilution of Detection Antibody in Preparation of Reagents and Materials.
Poor replicates	Insufficient washing	Ensure that each well is washed 5X with 200 µL of diluted Wash Buffer. Ensure that the multi-channel pipette fills and empties reproducibly. Blot wells dry before proceeding to the next step. If using an automated plate washer, add a 30-second soak period following the addition of wash buffer, and/or rotate the plate 180 degrees between wash steps.
	Unequal volumes in wells	Ensure that pipettes function properly.
	Carry-over between wells; evaporation from wells; splashing of well contents onto adhesive cover	Always use a new Adhesive Plate Cover for each incubation. Ensure that each well is sealed tightly. Keep filled plates in a horizontal position and handle with caution.
	Samples not mixed after thawing	Vortex samples after thawing.
	High concentration of lipids or particulate matter in samples	Filter or centrifuge samples to pellet aggregates. Lipids may concentrate on the surface after centrifugation and may be removed.
	Air bubbles in well during measurement with the ELISA reader	Remove air bubbles using a small hypodermic needle or pipette tip.
Absorbance values of sample dilutions decrease with increasing concentration	Protein concentration of the sample dilutions exceeds the upper limit of the assay	Dilute the samples further and retest along with the standard curve dilutions.

Related Products

For a complete list of ELISA kits and related products from STEMCELL Technologies, visit www.stemcell.com or contact us at techsupport@stemcell.com.