

Human anti-SARS-CoV-2 nucleoprotein(NP) IgG antibody ELISA Kit

Catalog No : EK7002XG

About This Assay

It is for the qualitative determination of anti-SARS-CoV-2 N protein-IgG in human serum, plasma.

Test Principle

The ELISA is based on the the qualitative enzyme immunoassay technique. The microplate provided in this kit has been pre-coated with human novel coronavirus nucleoprotein. Samples are pipetted into the wells with anti-human IgG antibody conjugated Biotin. Next, Streptavidin-HRP is added to each microplate well and incubated. After TMB Substrate solution is added, Any antibodies specific for the antigen present will bind to the pre-coated antigen. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of human novel coronavirus nucleoprotein (SARS-CoV-2 NP) IgG antibody bound in the initial step. The color development is stopped and the intensity of the color is measured.

Materials Supplied

Store as follows for Unopened kit, it is 12 months shelf life. Store at 4°C for opened kit, it is one month shelf life.

Reagents	96T Quantity/Size	48T Quantity/Size	Storage
Pre-coated assay plate	1 (96 wells)	1/2(48 wells)	-20°C
Positive Control	2 Vial	1 Vial	
Negative Control	2 Vial	1 Vial	
Biotin-antibody(100×)	1x 120 µ L	1x 60 µ L	
Streptavidin-HRP(100×)	1x 120 µ L	1x 60 µ L	
Universal Diluent	2x 20 mL	1x 20 mL	
TMB Substrate	1x 10 mL	1x 5 mL	4°C
Wash Buffer(30×)	2x 15 mL	1x 15 mL	
Stop Solution	1x 6 mL	1x 3 mL	
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Materials Needed But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water
7. 0.01M Phosphate Buffered Saline(PBS), pH 7.2-7.4

Samples Collection and Storage

Serum: Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 25 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 20 minutes at 1,000×g at 2-8 °C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze-thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 4 °C, otherwise samples must be stored at -20°C (≤6 months) or -80°C (≤12 months) to avoid loss of bioactivity and contamination.
2. Samples hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

Samples Preparation

1. Dilute the serum or plasma samples with Universal Diluent(1:100) before test. **The suggested 100-fold dilution can be achieved by adding 3 µL sample to 297 µL of Universal Diluent.**
2. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
3. Several trials may be necessary. The test sample must be well mixed with the

Universal Diluent. And also Positive Control (Negative Control) and samples should be making in pre-experiment.

4. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

Reagent Preparation

Bring all kit components and samples to room temperature (18-25 °C) before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

1. **Positive Control** and **Negative Control** - Dissolve them to the working concentration with 400 µL **Universal Diluent**.
2. **Biotin-antibody(1×)** and **Streptavidin-HRP(1×)** - Briefly spin or centrifuge the stock Biotin-antibody(100×) and Streptavidin-HRP(100×) before Dilution. Dilute them to the working concentration with **Universal Diluent**. A suggested 100-fold dilution is 10 µl of Biotin-antibody(100×) or Streptavidin- HRP(100×) + 990 µl of **Universal Diluent**.
3. **Wash Buffer(1×)** - Dilute 15 mL of Wash Buffer (30×) with 435 mL of deionized or distilled water to prepare 450 mL of Wash Buffer (1×).
4. **TMB Substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare **Positive Control** and **Negative Control** within 15 minutes before assay. Please do not dissolve the reagents at 37 °C directly.
3. Please carefully reconstitute **Positive Control** and **Negative Control** according to the instruction and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10 µL for one pipetting.
4. The reconstituted **Positive Control** and **Negative Control** can be used only once.
5. If crystals have formed in the Wash Buffer (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
6. Contaminated water or container for reagent preparation will influence the detection result.

Plate Set Up

The 96-wells plate included with this kit is supplied ready to use. It is not necessary to rinse the plate prior to adding the reagents.

A suggested plate format is shown in **Figure 1** below. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by AFG Bioscience .

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

PC=Positive Control; NC=Negative Control; BLK=Blank; S=Sample

Figure 1

Assay Procedure

1. Determine wells for **Positive Control** and **Negative Control**, blank and sample. Prepare Blank well without any solution.
2. Add 100 μ L of **Negative Control**, **Positive Control** or **diluted Sample** per well. Cover with the Plate sealer. Incubate for 30 minutes at 37 $^{\circ}$ C.
3. Aspirate each well and wash with 300 μ L of **Wash Buffer(1 \times)** to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
4. Add 100 μ L of **Biotin-antibody(1 \times)** to each well(not to blank). Cover with the Plate sealer. Incubate for 30 minutes at 37 $^{\circ}$ C.
5. Repeat the aspiration/wash process for total 3 times as conducted in step 3
6. Add 100 μ L of **Streptavidin-HRP(1 \times)** to each well(not to blank), Cover the wells with the plate sealer and incubate for 30 minutes at 37 $^{\circ}$ C.
7. Repeat the aspiration/wash process for total 5 times as conducted in step 3.

8. Add 90 μL of **TMB Substrate** to each well. Incubate for 15-20 minutes at 37 °C. Protect from light. The liquid will turn blue by the addition of **TMB Substrate**.
9. Add 50 μL of **Stop Solution** to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450 nm immediately.

Assay Procedure Summary

1. Prepare all reagents, samples and Negative Control, Positive Control.
2. Add 100 μL Negative Control, Positive Control or sample to each well. Incubate for 30 minutes at 37°C .
3. Aspirate and wash 3 times.
4. Add 100 μL **Biotin-antibody(1 \times)** to each well(not to blank). Incubate for 30 minutes at 37°C .
5. Aspirate and wash 3 times.
6. Add 100 μL **Streptavidin-HRP(1 \times)** to each well(not to blank). Incubate for 30 minutes at 37°C .
7. Aspirate and wash 5 times.
8. Add 90 μL **TMB Substrate**. Incubate for 15-20 minutes at 37°C .
- 9 Add 50 μL **Stop Solution** and read at 450 nm immediately.

Note:

1. **Assay preparation:** Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20 °C.
2. **Samples or reagents addition: Please use the freshly prepared Negative Control, Positive Control.** Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all **Negative Control, Positive Control** and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions

of **Negative Control**, **Positive Control**, samples, and reagents. Also, use separated reservoirs for each reagent.

3. **Incubation:** To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, do not let the strips dry at any time during the assay. Incubation time and temperature must be controlled.
4. **Washing:** The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.
5. **Controlling of reaction time:** Observe the change of color after adding **TMB Substrate**(e.g. observation once every 7 minutes), if the color is too deep, add **Stop Solution** in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
7. **TMB Substrate** is easily contaminated. Please protect it from light.

Precision

Intra-assay Precision (Precision within an assay): CV% < 10%

Three samples of known concentration were tested twenty times on one plate to assess.

Inter-assay Precision (Precision between assays): CV% < 10%

Three samples of known concentration were tested in twenty assays to assess.

Calculation Of Results

For calculation the valence of human novel coronavirus nucleoprotein (SARS-CoV-2 NP) IgG antibody, compare the sample well with control.

When $OD(\text{Positive Control}) \geq 0.5$ and $OD(\text{Negative Control}) \leq 0.15$, it represents the results are valid. If $OD(\text{sample})$ close to the Cut off value, we recommend repeating the experiment.

OD (blank) < 0.1

Cut off value = $OD(\text{Negative Control}) + 0.15$

Negative Result : $OD(\text{sample}) < \text{Cutoff value}$

Positive Result : $OD(\text{sample}) \geq \text{Cutoff value}$

Declaration

1. Limited by current conditions and scientific technology, we can't conduct comprehensive identification and analysis on all the raw material provided. So there might be some qualitative and technical risks for users using the kit.
2. Do not mix or substitute reagents from one kit to another. Use only the reagents supplied by us, please ask for our advice if you use any other reagents.
3. **This product is for research only, not for human or veterinary diagnostic or therapeutic use !**

Technical Service Contact information

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In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).