



ELISA PRODUCT INFORMATION & MANUAL

**Mouse SARS-CoV-2 Nucleocapsid
Antibody (Mouse IgG) ELISA Kit
(Colorimetric)**

NBP3-17987

Sample Insert for Reference Only

Enzyme-linked Immunosorbent Assay for quantitative
detection. For research use only.

Not for diagnostic or therapeutic procedures.

Intended Use

The kit applies for detecting the level of anti-SARS-CoV-2 Nucleocapsid Antibody (Mouse IgG) antibodies in mouse serum.

PRINCIPLE OF THE ASSAY

The principle of the kit is indirect ELISA. SARS-CoV-2 Nucleocapsid His Recombinant Protein has been pre-coated onto well plate strips. The samples are added to the well, after incubation the wells are washed and a horseradish peroxidase conjugated Rabbit anti-Mouse IgG is added, producing an complex "antigen-antibody-labeled antibody". Following a wash, the TMB substrate solution is loaded and color develops in proportion to the amount of antibodies. The reaction is stopped by the addition of a stop solution and the intensity of the color can be measured at 450 nm.

MATERIALS PROVIDED

Components	1 Kit (96 Tests)
Microplate: Pre-coated with SARS-CoV-2 Nucleocapsid His Recombinant Protein	1 Plate
HRP-Rabbit anti-Mouse IgG	250 μ L
20 \times Dilution Buffer	5 mL
anti-SARS-CoV-2 Nucleocapsid Antibody (Control)	20 μ L
20 \times Wash Buffer	25 mL
Color Reagent A	12.5 mL
Color Reagent B	12.5 mL
Stop Solution	8 mL

STORAGE

Unopened Kit	Store at 2 - 8°C and the kit is stable for 3 months upon receipt.	
Opened Reagents	HRP-Rabbit anti-Mouse IgG	Store for up to 1 month at 2 - 8°C
	anti-SARS-CoV-2 Nucleocapsid Antibody (Control)	Store for up to 1 month at 2 - 8°C
	Microplate	Return unused strips to the foil pouch containing the desiccant pack and reseal along entire edge of zip-seal. Store for up to 1 month at 2 - 8°C
	Diluted Dilution Buffer	Store for up to 1 month at 2 - 8°C
	Diluted Wash Buffer	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Stop Solution	

Please store the reagents as above conditions upon receiving, and used as soon as possible after opening.

REAGENT PREPARATION

Bring all reagents to room temperature before use. If crystals have formed in buffer solution, warm to room temperature and mix gently until the crystals have completely dissolved.

Wash Buffer - Prepare 1×Wash Buffer by adding 15 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 300 mL of Wash Buffer.

Dilution Buffer - Prepare 1×Dilution Buffer by adding 5 mL of Dilution Buffer Concentrate to deionized or distilled water to prepare 100 mL of Dilution Buffer.

Notes: 1× dilution buffer is blank, and each tube of sample is fully mixed before further dilution.

HRP-Rabbit anti-Mouse IgG - 1:60 to 1:500 dilute with 1×dilution buffer 10 minutes before use.

anti-SARS-CoV-2 Nucleocapsid Antibody (Control) - 1:25000-1:100000 dilute with 1×dilution buffer 10 minutes before use.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 10 minutes of use. **Take care not to contaminate the Color Reagent. If the mixed color reagent is blue. DO NOT USE.**

ASSAY PROCEDURE

1. Remove unused microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Wash each well five times with 1×Wash Buffer (300 µL/well, immersion 1 minutes) using multi-channel pipette, manifold dispenser or autowasher.
Complete removal of liquid at each step is essential to good performance. Remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
3. Add 100 µL antibody control or your test sample per well. The recommended dilution of the sample is 1:10000 to 1:600000 or higher dilution (Optimal concentrations/dilutions should be determined by the end user), **Ensure reagent addition is uninterrupted and completed within 15 minutes.** Cover/seal the plate and incubate for 2 hours at room temperature
4. Removal the liquid in the wells and repeat the aspiration/wash as in Step 2.
5. Dilute HRP-Rabbit anti-Mouse IgG 1:60 to 1:500 in 1×Dilution Buffer. Add 100 µL into each well, mix gently. Cover/seal the plate and incubate for 1 hour at room temperature.
6. Removal the liquid in the wells and repeat the aspiration/wash as in Step 2.
7. Add 200 µL of Substrate Solution to each well, mix gently. Incubate for 20 minutes at room temperature. **Protect from light.** (According to the color of sample and the control antibody, the chromogenic time should be shortened or prolonged.)
8. Add 50 µL of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. **Determine the optical density of each well within 20 minutes**, using a microplate reader set to 450 nm.

PRECAUTIONS

1. This kit is **for research use only** and is not for use in diagnostic or therapeutic procedures.
2. The kit should not be used beyond the expiration date.
3. Do not mix reagents from different lots.
4. The kit is designed and tested to detect the application which shown in the manual. The use of this kit for other purpose should be verified carefully by the end user.

SAFETY INSTRUCTIONS

1. The Stop Solution provided with this kit is an acid solution. Take care when using the reagent to avoid the risks.
2. All biological materials should be handled and discarded as potentially hazardous following local laws and regulations.
3. Personal protective equipments such as lab coats, gloves, surgical masks and goggles are necessary in experiments for safety reasons.

TECHINICAL TIPS

1. Bring all reagents and samples to room temperature before use.
2. Samples should be thawed completely and mixed well prior to analysis. Avoid repeated freeze-thaw cycles of frozen samples.
3. Use a new disposable reagent reservoir and new disposable pipette tips for each transfer to avoid cross-contamination.
4. Read the absorbance of each well within 20 minutes after adding the stop solution.