ABclonal Technology

SARS-CoV-2 Nucleocapsid Protein Uncoated ELISA Kit

Catalog NO.:RK04546

Product introduction

This product uses sandwich ELISA method to detect natural and recombinant SARS-Nucleocapsid. The recommended reagent is suitable for most cell culture supernatant, serum and plasma samples. The optimized reagent can change the performance of immunoassay, and the dilution optimization of samples containing complex matrix (such as serum and plasma), such as serum and plasma, can improve the detection effect in this experiment.

***This product is only used for scientific research and can not be used for clinical diagnosis.** Before using this product, please be sure to read the instructions in the product package.

Components of the kit and preservation conditions

The unopened kit shall be stored at 2-8 ℃ for no more than 2 months. If it needs to be stored for a long time, please store it at - 20 ℃. Do not use expired components.

Reagent	Reagent specification	Reagent article No	Quantity		Working concentration	Reagent storage
			10 plates	20 plates		
SARS-CoV-2 Nucleocapsid Protein Capture Antibody	150ug/vial	RM95240	1 vial	2 vials	1-4ug/ml	Can be stored at -20 ℃ for up to 12 months.
SARS-CoV-2 Nucleocapsid Protein Biotin-Conjugate Antibody	7.5ug/vial	RM95242	1 vial	2 vials	50-200ng/ml	
SARS-CoV-2 Nucleocapsid Protein Standard Lyophilized	100ng/vial	RM95241	1 vial	2 vials	7.8-500pg/ml	
Streptavidin-HRP Concentrated (200×)	700μL/vial	RM60000	1 vial	2 vials	200X	

Other required reagents (Uncoated Ancillary Reagent Kits: RK04587)

It can also be purchased separately.

Components	Specifications	货号	Reagent storage
Coating Buffer	1 x 20 mL	RM01756	Can be stored at 2-8 ℃ for up to 6 months.
Blocking Buffer	1 x 30 mL	RM01757	
Standard/Sample Diluent (R1)	1 x 20 mL	RM00023	
Biotin-Conjugate Antibody Diluent (R2)	1 x 12 mL	RM00024	
Streptavidin-HRP Diluent (R3)	1 x 12 mL	RM00025	
Wash Buffer(20x)	1 x 30 mL	RM00026	
TMB Substrate	1 x 12 mL	RM00027	
Stop Solution	1 x 6 mL	RM00028	
Plate Sealers	4 x 1pcs	RM01759	Can be stored at room temperature for up to 6 months
uncoated plate	1 x 96T	RM01758	

***It's recommended to diluent Wash Buffer(20x) with Ultra-pure water.**

Some components list above may be preparation separately:

1. **Coating buffer(PBS):** 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na2HPO4, 1.5 mM KH2PO4, pH 7.2-7.4, 0.2 μm filtered.

2. **Blocking Buffer:** 1% BSA, 5% sucrose in PBS, pH 7.2-7.4, 0.2 μm filtered.
3. **Standard/Sample Diluent (R1) /Biotin-Conjugate Antibody Diluent(R2)/Streptavidin-HRP Diluent(R3):** 1% BSA in PBS, pH 7.2-7.4, 0.2 μm filtered.
4. **Wash Buffer:**0.05% Tween® 20 in PBS, pH 7.2-7.4
5. **TMB Substrate:**1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (Tetramethylbenzidine) .
6. **Stop Solution:**1 N HCL。

Other Supplies Required

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 570 nm.
2. Multi range single/multi-channel pipette and pipette tips.
3. Deionized or distilled water.
4. Squirt bottle, manifold dispenser, or automated microplate washer.
5. Incubator.
6. Test tubes for dilution of standards and samples.

Precautions

1. Please pay attention to the expiration date on the product label and use this product before the expiration date.
2. Do not mix reagents of different batches or from different manufacturers.
3. Sample adding, plate washing, incubation time, incubation temperature and other operations during the experiment will affect the final results. Please strictly manage the experiment process and make records.
4. The collection, processing and storage of samples will have an impact on the test results. Please strictly manage the sample processing process.
5. The possibility of interference cannot be ruled out before all factors are tested in this test.
6. There may be harmful substances in relevant reagents, which may cause skin allergic reaction. Avoid inhalation. Please wear gloves and goggles when necessary.
7. To ensure the best detection effect, please refer to the label or instructions when storing or using the relevant reagent components, and pay attention to avoiding light.
8. Evenly mixing the reagent after preparation is very important to the test results, but some proteins or antibodies may be very sensitive to severe vortex, which may cause loss of activity. Please use vortex with caution.
9. In the process of reagent preparation and liquid transfer, please use sterilized consumables and pay attention to replacement to avoid reagent contamination and affecting the final test results.
10. To ensure the best detection effect, it is not recommended to reuse the working solution of dissolved standard protein and related reagents after freezing.
11. Please prepare reagents in strict accordance with the instructions.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing the correlated IgG as in this kit may interfere with this assay.

1. **Cell Culture supernatants:** Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
2. **Serum :** Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
3. **Plasma :** Collect plasma using EDTA or Heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g within 30 minutes of coolection. Assay immediately or aliquot and store samples at ≤ -20℃. Avoid repeated freeze-thaw cycles. (Note: Citrate plasma has not been validated for use in this assay.)
4. **Note :** It is suggested that all samples in a study be collected at the same time of the day.Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

Reagent Preparation

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

1. **Capture Antibody:**

① Add 1mL PBS into the freeze-dried product and mix gently for at least 15min,According to the experimental requirements, The remaining Capture Antibody is packaged and stored at -20 to -70℃ to avoid repeated freezing and thawing.

② Capture Antibodies were diluted in Coating buffer to the working concentration，Add 100 μL of the diluted Capture Antibody to a uncoated plate each well. Incubate overnight at 2-8℃.

③ Discard the liquid in the hole, Blot plate on absorbent paper to remove any residual buffer, adding 200 μL of Blocking Buffer to each well. Incubate for a minimum of 1 hour at 37℃.

④ Discard the liquid in the hole, Blot plate on absorbent paper to remove any Blocking Buffer, and vacuum them with aluminum foil bags.

2. **Standard:**

① Add 1mL PBS or Standard/Sample Diluent（R1） into the freeze-dried product and mix gently for at least 15min, According to the experimental requirements, The remaining standard is packaged and stored at -20 to -70℃ to avoid repeated freezing and thawing.

② According to the standard curve of the concentration degree of ladder dilution (recommended standard curve using the following concentrations: 500, 250, 125,62.5, 31.2, 15.6, 7.8, 0pg/mL).The prepared standard is used within 20 minutes.

3. **Biotin-Conjugate Antibody：**

① Add 1mL PBS into the freeze-dried product and mix gently for at least 15min,According to the experimental requirements,The remaining Biotin-Conjugate Antibody is packaged and stored at -20 to -70℃ to avoid repeated freezing and thawing.

② Use the Biotin-Conjugate Antibody Diluent（R2） to dilute the antibody to the working concentration, and use it according to the recommended working concentration for antibody detection. Note: The diluted working solution should be used within 30 minutes.

4. **Streptavidin-HRP Concentrated(200x)** : It is recommended to dilute 1:200 of Concentrated Streptavidin-HRP (200x) with Streptavidin-HRP Diluent (R3) before use.Note: The diluted working solution should be used within 30 minutes.

Assay Procedure

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

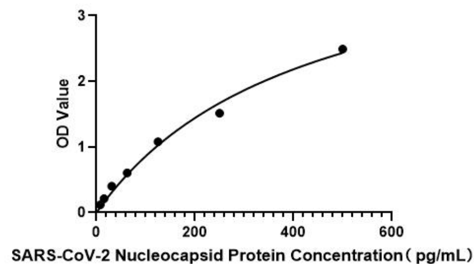
1. Prepare all reagents, working standards, and samples as directed in the previous sections. Prepare the SARS-CoV-2 Nucleocapsid Protein Biotin-Conjugate Antibody Working Solution and the Streptavidin-HRP Concentrated (200X) Working Solution 15minutes early before use.
2. Add wash buffer 350 μL/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes, After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Add 100 μL Standard/sample Diluent (R1) in a blank well.Add 100 μL different concentration of standard or sample in other wells, Cover with the adhesive strip provided. Incubate for 2 hours at 37℃. record the plate layout of standards and sample assay.
4. Repeat the aspiration/wash as in step 2.
5. Add 100 μL/well diluted Biotin-Conjugate Antibody to all wells. cover with new adhesive strip provided. Incubate for 1 hour at 37℃.
6. Repeat the aspiration/wash as in step 2.
7. Add 100 μL of the working dilution of Streptavidin-HRP to each well. Cover the plate and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.
8. Repeat the aspiration/wash as in step 2.
9. Add 100 μL TMB Substrate to each well. Incubate for 15-20 minutes at 37℃ .Protect from light.
10. During the incubation, turn on the microplate reader to warm up.
11. Add 50 μL Stop Solution, determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

Calculation of Results

1. Average the duplicate readings for each standard, control and sample, andsubtract the average zero standard optical density (O.D.).
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve. The data may be linearized by plotting the Nucleocapsid concentrations versus the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical Data

The standard curves are provided for demonstration only. A standard curve should be generated for each set of Nucleocapsid assayed.



Specificity

The test recognizes recombinant and natural SARS-CoV-2 Nucleocapsid Protein.

Trouble Shooting

Problem	Possible Cause	Solution
High Background	Insufficient washing	Sufficiently wash plates as required. Ensure appropriate duration and number of washes. Ensure appropriate volume of wash buffer in each well.
	Incorrect incubation procedure	Check whether the duration and temperature of incubation are set up as required.
	Cross-contamination of samples and reagents	Be careful of the operations that could cause cross-contamination. Use fresh reagents and repeat the tests.
No signal or weak signal	Incorrect use of reagents	Check the concentration and dilution ratio of reagents. Make sure to use reagents in proper order.
	Incorrect use of microplate reader	Warm the reader up before use. Make sure to set up appropriate main wavelength and correction wavelength.
	Insufficient colour reaction time	Optimum duration of colour reaction should be limited to 15-25 minutes.
	Read too late after stopping the colour reaction	Read the plate in 5 minutes after stopping the reaction.
	Matrix effect of samples	Use positive control.
Too much signal	Contamination of TMB substrate	Check if TMB substrate solution turns blue. Use new TMB substrate solution.
	Plate sealers reused	Use a fresh new sealer in each step of experiments.
	Protein concentration in sample is too high	Do pre-test and dilute samples in optimum dilution ratio.
Poor Duplicates	Uneven addition of samples	Check the pipette. Periodically calibrate the pipette.
	Impurities and precipitates in samples	Centrifuge samples before use.
	Inadequate mixing of reagents	Mix all samples and reagents well before loading.