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SARS-CoV-2 Neutralizing Antibody ELISA Kit

(Omicron BA.1 / BA.2 / BA.4 / BA.5)

(For Research Use Only. Not for Use in Diagnostic or Therapeutic Applications)

GTX537233

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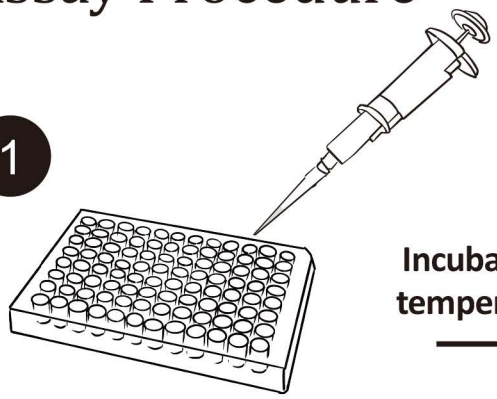
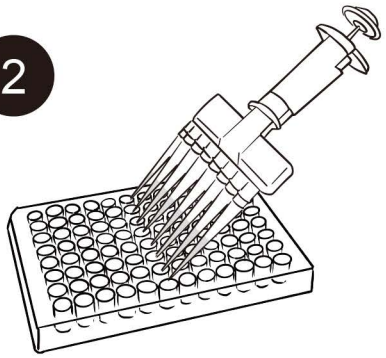
Assay Procedure

1

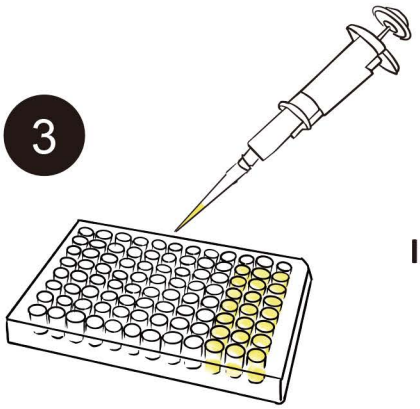


Add 25 µl of PC, NC, or sample solutions to designated wells. Then add 25 µl 2× spike protein solution to all wells. Mix gently.

Incubate covered at room temperature for 1.5 hours.

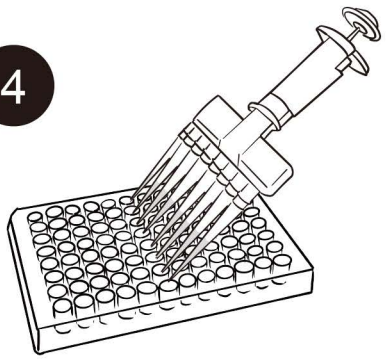


Aspirate and wash each well six times.

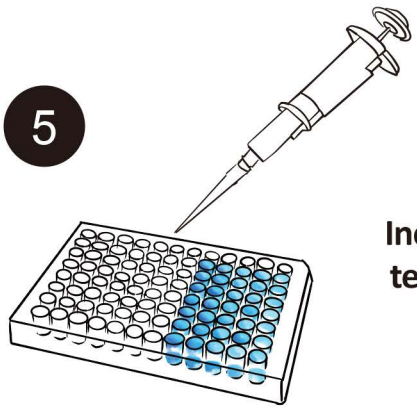


Add 50 µl 1× Conjugate Solution to each well.

Incubate covered at room temperature for 1 hour.

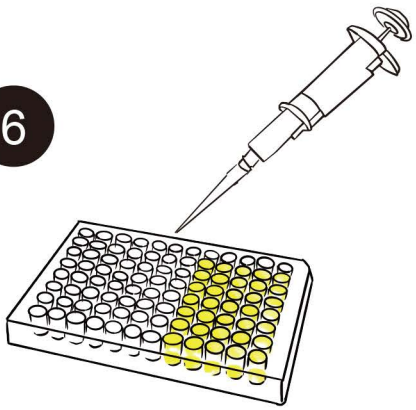


Aspirate and wash each well six times.

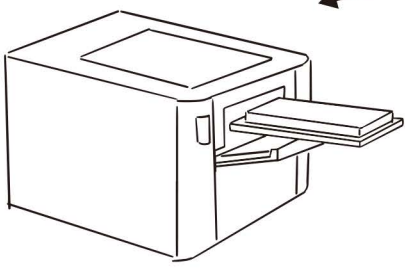


Add 100 µl TMB Solution to each well.

Incubate in darkness at room temperature for 15 minutes.



Add 100 µl Stop Solution to each well.



Measure absorbance of each well at 450 nm (within 15 minutes).

Table of Contents

1. Introduction

SARS-CoV-2 infects host cells through binding of its spike protein's receptor-binding domain (RBD) to angiotensin-converting enzyme 2 (ACE2) on the host cell surface. This interaction can be inhibited by neutralizing antibodies (NAb). Detection of NAb in convalescent or vaccinated sera is important to assess protection from reinfection. The GeneTex SARS-CoV-2 Neutralizing Antibody ELISA Kit (Omicron BA.1 / BA.2 / BA.4/5) (GTX537233) is an in vitro assay for qualitative SARS-CoV-2 neutralizing antibody (NAb) screening. The kit includes recombinant His-tagged SARS-CoV-2 trimeric spike proteins for the wildtype (WT) strain, Omicron variant BA.1, Omicron variant BA.2, and Omicron variant BA.4/5, for Omicron variant-specific neutralization analysis.

The assay methodology is straightforward. Recombinant ACE2 protein is immobilized in the wells of the included 96-well plate (12 x 8-well strips). Control antibody (either NAb or Non-NAb) mixtures or samples are pipetted into the appropriate wells, followed by addition of the recombinant His-tagged spike protein(s). Neutralizing agents in the samples (or in the NAb Control Antibody solution) react with spike proteins to prevent them from binding to ACE2. After washing away unbound proteins and antibodies, an HRP-conjugated anti-His tag antibody is added to bind to the immobilized recombinant spike proteins. The wells are washed again to remove free conjugated antibody, and TMB substrate is added for color development. The Stop Solution changes the color from blue to yellow, and the intensity of the color (measured at 450 nm) inversely correlates with the titer of the anti-spike neutralizing antibodies.

2. Storage

The unopened kit can be stored at 2-8°C for up to 6 months.

3. Reagents

Item	*Name*	*Description*	*Quantity*	*Stability (at 2-8°C)*
A	ACE2 Microplate	Coated with recombinant ACE2 protein	96 wells, 1 plate (12 x 8-well strips)	1 month after opening *
B1	HRP-Conjugated Anti-His Tag Antibody	Horseradish peroxidase-conjugated mouse monoclonal antibody; Preservative: Proclin 300 (0.05%)	20 µl, 1 vial	1 month after preparation
B2	1× Conjugate Solution		N/A	4 hours after preparation
C1	Assay Diluent	This solution is used to dilute HRP-Conjugated Anti-His Tag Antibody (Item B1) and recombinant spike proteins (Items E1-E4); Preservative: Proclin 300 (0.05%)	12 ml, 2 bottles	1 month after opening
C2	Control Diluent	This solution is used to dilute Control Antibodies (Items D1 and D2) and test serum samples (when necessary); Preservative: Proclin 300 (0.05%)	10 ml, 1 bottle	1 month after opening
C3	Reconstitution Solution	This solution is used to reconstitute lyophilized Control Antibodies (Items D1 and D2) and spike proteins (Items E1-E4). Preservative: Proclin 300 (0.05%)	1 ml, 1 vial	1 month after opening
D1	NAb Control (Lyophilized)	Mixture of rabbit monoclonal antibodies against the SARS-CoV-2 spike protein with strong neutralizing capability	1 vial	12 hours after preparation
D2	Non-NAb Control (Lyophilized)	Rabbit monoclonal antibody against the SARS-CoV-2 spike protein with non-neutralizing capability	1 vial	
E1	Spike WT (Lyophilized)	Purified recombinant SARS-CoV-2 spike wild-type (WT) protein, His tag	1 vial	
E2	Spike Omicron BA.1 (Lyophilized)	Purified recombinant SARS-CoV-2 spike protein, BA.1 / Omicron variant, His tag	1 vial	
E3	Spike Omicron BA.2 (Lyophilized)	Purified recombinant SARS-CoV-2 spike protein, BA.2 / Omicron variant, His tag	1 vial	
E4	Spike Omicron BA.4/5 (Lyophilized)	Purified recombinant SARS-CoV-2 spike protein, BA.4/5 / Omicron variant, His tag	1 vial	
F1	20× Wash Buffer	Tween-20, PBS buffer	50 ml, 2 bottles	1 month after opening
F1	20× Wash Buffer		N/A	1 week after preparation
G	TMB Solution	3,3',5,5'-tetramethylbenzidine (TMB)	10 ml, 2 bottles	1 month after opening
H	Stop Solution	1 N Sulfuric acid	10 ml, 2 bottles	

* *Unused plate strips should be resealed securely in the included aluminum foil zip-lock bag containing a desiccant and stored at 2-8°C.*

4. Additional Materials Required (Not provided)

4.1. Pipettes capable of delivering 10 µl, 200 µl, and 1000 µl volumes.
4.2. Disposable pipette tips suitable for the pipettes indicated above.
4.3. Deionized or distilled water to dilute 20× Wash Buffer.
4.4. Microplate multichannel automatic or semi-automatic washing system (optional).
4.5. Spectrophotometric microplate reader capable of measuring absorbance at 450 nm.
4.6. Automated ELISA analyzer (Dynex, DS2, optional).
4.7. Aluminum foil (optional).

5. Sample Collection and Storage

5.1. Serum: Collect whole blood and allow blood to clot for 30 minutes at room temperature, then centrifuge for 15 minutes at 1000-2000xg. Transfer the supernatant (serum) immediately to a clean polypropylene tube. Store samples ≤ −20°C. Aliquot to avoid freeze-thaw cycles.

6. Reagent Preparation

6.1. Bring all reagents and samples to room temperature (18-25°C) before use.
*6.2. Reconstitution of neutralizing NAb Control (**Item D1**) and non-neutralizing Non-NAb Control (**Item D2**): Briefly spin vials to ensure all contents are at the bottom. Add 50 µl Reconstitution Solution (**Item C3**) to D1 vial and to D2 vial to prepare 200× NAb and 200x Non-NAb Controls, respectively. Resuspend thoroughly with gentle pipetting. Store the reconstituted NAb and Non-NAb Control Antibodies at −20°C. Aliquot to avoid multiple freeze-thaw cycles.*
*6.3. Preparation of working Positive Control (PC) and Negative Control (NC) solutions: Add 30 µl of 200× NAb Control or 200× Non-NAb Control (see step 6.2) to 2970 µl Control Diluent (**Item C2**) (i.e., a 100-fold dilution) to prepare PC solution (2× NAb Control) and NC solution (2× Non-NAb Control), respectively.*
*6.4. Reconstitution of recombinant spike proteins (**Items E1** to **E4**): Briefly spin the vials to ensure all contents are at the bottom. Add the amount of Reconstitution Solution (**Item C3**) shown on each vial label to prepare a 1000× spike protein stock for each protein. Resuspend thoroughly with gentle pipetting. The reconstituted spike proteins are stable for 1 month at −20°C. Aliquot to avoid multiple freeze-thaw cycles.*
*6.5. Preparation of working 2× spike protein solutions for 24 tests: Add 6 µl of the required 1000x spike protein stock (see step 6.4) to 2994 µl Assay Diluent (**Item C1**) (i.e., a 500-fold dilution) to prepare a 2× spike protein solution.*

6.6. *Sample dilution (optional): Control Diluent (**Item C2**) should be used for dilution of high-titer serum samples. Note: The titer of SARS-CoV-2 neutralizing antibodies may vary among samples. Optimal dilution factors for each sample must be determined empirically by the investigator.*

6.7. *If crystallization occurs in the 20× Wash Buffer (**Item F1**), warm the buffer to 37°C and mix gently until dissolved. Dilute 50 ml of 20× Wash Buffer into 950 ml deionized water (i.e., a 20-fold dilution) to yield 1000 ml of 1× Wash Buffer (**Item F2**).*

6.8. *Preparation of HRP-Conjugated Anti-His Tag Antibody (**Item B1**):*
*Briefly spin the vial to ensure all contents are at the bottom. Add 330 µl Assay Diluent (**Item C1**) and gently pipette to mix. The diluted HRP-Conjugated Anti-His Tag Antibody solution is stable for 1 month at 4°C.*

6.9. *Preparation of working 1× Conjugate Solution (**Item B2**): The diluted HRP-Conjugated Anti-His Tag Antibody solution (see step 6.8) should be freshly diluted 40-fold with Assay Diluent (**Item C1**) before use in step 7.6 of "Assay Procedure". For example, to prepare a working 1× Conjugate Solution sufficient for 96 tests, add 200 µl diluted HRP-Conjugated Anti-His Tag Antibody solution to 7800 µl of Assay Diluent. Mix gently by pipetting.*

7. Assay Procedure

7.1. *Bring all reagents and samples to room temperature (18-25°C) before use.*

7.2. *Place the appropriate number of 8-well strips from the plate in a holder. Label the 8-well strips according to the experimental design. It is recommended that all controls and samples be run in duplicate to reduce variation within the assay.*

7.3. *Add 25 µl of working Positive Control (PC solution) and Negative Control (NC solution) (see "Reagent Preparation" steps 6.2 and 6.3) to the respective "PC" and "NC" wells. Add 25 µl of each test sample (see "Reagent Preparation" step 6.6) into the designated wells. See possible experimental design configurations below.*

Configuration Option I *(Four spike proteins tested: two controls (in duplicate) and 20 samples per spike protein)*

	WT			*Omicron BA.1*			*Omicron BA.2*			*Omicron BA.4/5*		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	NC	S5	S13	NC	S5	S13	NC	S5	S13	NC	S5	S13
B	NC	S6	S14	NC	S6	S14	NC	S6	S14	NC	S6	S14
C	PC	S7	S15	PC	S7	S15	PC	S7	S15	PC	S7	S15
D	PC	S8	S16	PC	S8	S16	PC	S8	S16	PC	S8	S16
E	S1	S9	S17	S1	S9	S17	S1	S9	S17	S1	S9	S17
F	S2	S10	S18	S2	S10	S18	S2	S10	S18	S2	S10	S18
G	S3	S11	S19	S3	S11	S19	S3	S11	S19	S3	S11	S19
H	S4	S12	S20	S4	S12	S20	S4	S12	S20	S4	S12	S20

Configuration Option II *(Four spike proteins tested: two controls (in duplicate) and 10 samples (in duplicate) per spike protein)*

	WT			*Omicron BA.1*			*Omicron BA.2*			*Omicron BA.4/5*		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	NC	S3	S7	NC	S3	S7	NC	S3	S7	NC	S3	S7
B	NC	S3	S7	NC	S3	S7	NC	S3	S7	NC	S3	S7
C	PC	S4	S8	PC	S4	S8	PC	S4	S8	PC	S4	S8
D	PC	S4	S8	PC	S4	S8	PC	S4	S8	PC	S4	S8
E	S1	S5	S9	S1	S5	S9	S1	S5	S9	S1	S5	S9
F	S1	S5	S9	S1	S5	S9	S1	S5	S9	S1	S5	S9
G	S2	S6	S10	S2	S6	S10	S2	S6	S10	S2	S6	S10
H	S2	S6	S10	S2	S6	S10	S2	S6	S10	S2	S6	S10

7.4. Add 25 µl of each working 2× spike protein solution (see "Reagent Preparation" steps 6.4 and 6.5) to the control and sample wells. Gently pipette in the well or tap the plate to mix. Cover wells with sealing film or a microplate lid. Incubate at room temperature for 1.5 hours.

7.5. Aspirate the solution in the wells. Wash by dispensing 300 µl 1× Wash Buffer (see "Reagent Preparation" step 6.7) into each well and then completely aspirate the buffer. Do this four to six times in total. Alternatively, an automatic washing system can be used. After the final wash, invert the plate and tap it on paper towels to remove residual buffer.

7.6. Add 50 µl of working 1× Conjugate Solution (see "Reagent Preparation" step 6.8 and 6.9) to each well. Cover the wells and incubate at room temperature for 1 hour.

7.7. Repeat step 7.5.

7.8. Add 100 µl of TMB Solution (Item G) to each well. Cover the wells (optional) and incubate in the dark (or cover with foil) for 15 minutes at room temperature.

7.9. Add 100 µl of Stop Solution (Item H) to each well. Read the optical density at 450 nm within 15 minutes.

8. Calculation of Results

Calculate the average absorbance values for each set of duplicated controls. If duplicated tests are performed for all samples, calculate the average absorbance value of the two readings.

8.1. ***Data Analysis:***

Calculate inhibition rate using the following formula:

Inhibition Rate (IR) = [1 - (average absorbance value of tested sample / average absorbance value of NC)] × 100%

8.2. ***Quality Control:***

If the data obtained from the controls do not meet the requirements shown below, repeating the assay is suggested. (See Section 10 "Troubleshooting Guide"):

Average absorbance value of "NC" ≥ 1.2

Inhibition Rate of "PC" ≥ 30%

8.3. ***Interpretation of Inhibition Rate:***

IR ≥ 30%: Neutralizing effect is present.

IR < 30%: Neutralizing effect is not present.

9. Technical Data

9.1. ***Precision***

Intra-Assay Coefficient of Variability (CV) (%) < 10%

Four samples (mixture of spike proteins and NAb (20 replicates)) were tested on one plate to assess intra-assay precision.

Inter-Assay CV (%) < 15%

Four samples (mixture of spike protein and NAb (4 replicates)) were tested in ten separate assays to assess inter-assay precision.

9.2. ***Clinical performance***

A total of 115 serum samples collected from 75 unvaccinated healthy individuals and 40 vaccinated healthy individuals were analyzed using the GeneTex SARS-CoV-2 Neutralizing Antibody ELISA Kit (Omicron BA.1 / BA.2 / BA.4/5). The detection of neutralizing antibody (NAb) against the WT strain was compared with that of a commercial ELISA kit.

		Comparator		
		Positive	**Negative**	**Total**
Neutralizing Antibody ELISA Kit (GTX537233) wild-type	**Positive**	36	0	36
	Negative	0	79	79
	Total	36	79	115
Positive Agreement		100% (95% CI: 90.4-100.0%)		
Negative Agreement		100% (95% CI: 95.4-100.0%)		

In addition to the results for the WT strain, five positive and five negative serum samples were further tested against SARS-CoV-2 Omicron virus (Omicron BA.1, BA.2 and BA.5 variants).

#	NAb against WT IR (%)	NAb against Omicron BA.1 IR (%)	Omicron BA.1		NAb against Omicron BA.2 IR (%)	Omicron BA.2		NAb against Omicron BA.4/5 IR (%)	Omicron BA.5	
			NT ($TCID_{50}$)	NT_{50} (IU/mL)		NT ($TCID_{50}$)	NT_{50} (IU/mL)		NT ($TCID_{50}$)	NT_{50} (IU/mL)
1	97.1	93.3	20	95.8	94.2	80	442.5	93.9	40	211.4
2	97.1	86.4	10	38.0	90.4	40	211.4	91.0	40	211.4
3	97.2	88.8	20	95.8	91.8	40	211.4	90.7	30	153.6
4	97.1	95.9	30	153.6	96.8	120	673.7	95.7	40	211.4
5	96.7	89.5	10	38.0	91.4	40	211.4	91.2	40	211.4
6	-4.1	0.4	<5	-0.2	-0.2	<5	1	-1.9	<5	1
7	-3.6	3.5	<5	-2.7	-2.7	<5	1	-1.8	<5	1
8	0.4	-3.5	<5	0.5	0.5	<5	1	2.1	<5	1
9	-3.3	0.1	<5	0.3	0.3	<5	1	2.3	<5	1
10	-5.6	-3.3	<5	-4.0	-4.0	<5	1	-1.0	<5	1

* *NT: neutralization titer; TCID50: 50% Tissue Culture Infectious Dose; NT50: 50% neutralization titer*

The virus neutralization test results were consistent with the results generated by the GeneTex SARS-CoV-2 Neutralizing Antibody ELISA Kit.

10. Troubleshooting Guide

Problem	Possible Cause	Solution
High signal in "PC" wells	Improper spike protein or NAb Control preparation	Check pipettes. See steps 6.2, 6.3, 6.4 and 6.5.
	Improper storage conditions	Aliquot and store reconstituted NAb Control at −20°C. Avoid freeze-thaw cycles.
Low signal in "NC" wells	Improper spike protein preparation	Check pipettes. See steps 6.2, 6.3, 6.4 and 6.5.
	Improper storage conditions	Aliquot and store reconstituted spike proteins at −20°C. Avoid freeze-thaw cycles.
	Improper dilution of conjugate solution	Check pipettes and see steps 6.8 and 6.9.
Large CV	Inaccurate pipetting	Check pipettes.
	Excessive residual wash buffer in wells	After final wash steps, invert the plate and tap it on clean paper towels to remove residual buffer. See step 7.5.