SARS-CoV-2 Neutralizing Antibody ELISA Kit

Catalog Number BMS2326 (96 tests), BMS2326TEN (10 × 96 tests)

Pub. No. MAN0024968 Rev. B.0 (31)

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The SARS-CoV-2 Neutralizing Antibody ELISA Kit is a competitive Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect the level of SARS-CoV-2 Neutralizing Antibody in serum and plasma. The 96-well plate is coated with a SARS-CoV-2 Receptor Binding Domain (RBD) antigen. Samples with neutralizing antibodies compete with excess amounts of biotinylated ACE2. Any ACE2 that binds to the RBD will produce signal. Signals are inversely proportional to the level of neutralizing antibodies.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No.	
	BMS2326 (96 tests)	BMS2326TEN (10 × 96 tests)
SARS-CoV-2 Neutralizing Ab Control, lyophilized	2 vials	10 vials
SARS-CoV-2 Neutralizing Ab Coated Plate	1 plate	10 plates
SARS-CoV-2 Neutralizing Ab Biotin Conjugate (100X), lyophilized	1 vial	10 vials
Assay Buffer Concentrate (20X)	5 mL	10 × 5 mL
Streptavidin-HRP Conjugate (100X)	0.150 mL	10 × 0.150 mL
Wash Buffer Concentrate (20X)	50 mL	6 × 50 mL
Stabilized Chromogen (Tetramethylbenzidine)	15 mL	10 × 15 mL
Stop Solution	15 mL	100 mL
Plate Covers, adhesive	4	40

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm, 490 nm, and 650 nm (polychromatic reading)
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Horizontal microplate shaker capable of 700 rpm ± 100 rpm
- Magnetic stirrer

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at thermofisher.com.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.
- For small-volume tubes, make sure to centrifuge the tubes before use to ensure pellets are not adhered to the cap.
- Volumes shown are for full-plate assays. If not using a full plate, divide volumes proportionately.

Sample preparation guidelines

- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.

Prepare 1X Wash Buffer

1. Dilute 25 mL of Wash Buffer Concentrate (20X) with 475 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 30 days.

Prepare 1X Assay Buffer

1. Dilute 5 mL of Assay Buffer (20X) with 95 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store 1X Assay Buffer at 2–8°C. The diluted buffer is stable for 30 days.

Pre-dilute samples

Note: Perform sample dilutions with 1X Assay Buffer.

Dilute serum and plasma samples 1:50 with 1X Assay Buffer (e.g., 5 µL of sample with 245 µL of 1X Assay Buffer).

Reconstitute control

The Neutralizing Ab Control is used as a positive control.

1. Reconstitute lyophilized control with 1X Assay Buffer. See control vial label for reconstitution volume.
2. Swirl or mix gently, then incubate for 10 minutes. **Use the control within 15 minutes of reconstitution.**

Prepare 1X Biotin Conjugate

Note: Reconstitute Biotin Conjugate 15 minutes prior to usage.

1. Reconstitute lyophilized Biotin Conjugate (100X) with 1X Assay Buffer. See control vial label for reconstitution volume.
2. Dilute 0.12 mL of reconstituted Biotin Conjugate (100X) with 11.88 mL of 1X Assay Buffer. Mix thoroughly.

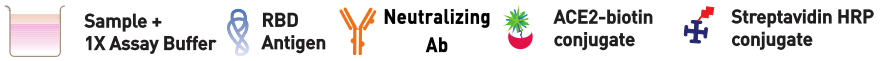
Prepare 1X Streptavidin-HRP conjugate solution

Note: Prepare 1X Streptavidin-HRP conjugate solution within 15 minutes of usage.

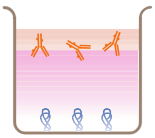
For each coated plate, pipet 0.12 mL of Streptavidin-HRP Conjugate (100X) and dispense the solution into a tube containing 11.88 mL of 1X Assay Buffer. Mix thoroughly.

Perform Assay (Total assay time: 1.5 hours)

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Perform positive and negative controls in duplicate with each assay. Negative controls are needed for calculations.

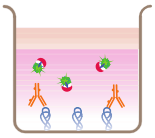


1 Bind antibody



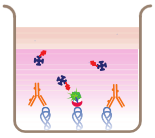
1.1. Wash wells 2 times with 1X Wash Buffer.
1.2. Add 100 µL of positive control or pre-diluted samples (see "Pre-dilute samples" on page 1) to the appropriate wells.
1.3. Add 100 µL of 1X Assay Buffer to wells as negative controls.
1.4. Cover the plate with a plate cover and incubate for 30 minutes at room temperature (20–25°C) **with shaking**.
1.5. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer. **Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration**.

2 Add 1X Biotin Conjugate



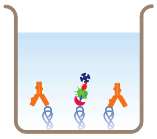
2.1. Add 100 µL of 1X Biotin Conjugate solution into each well.
2.2. Cover the plate with a plate cover and incubate for 30 minutes at room temperature (20–25°C) **with shaking**.
2.3. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer. **Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration.**

3 Add 1X Streptavidin-HRP Conjugate



3.1. Add 100 µL of 1X Streptavidin-HRP Conjugate solution into each well.
3.2. Cover the plate with plate cover and incubate for 15 minutes at room temperature (20–25°C) **with shaking**.
3.3. Thoroughly aspirate the solution and wash wells 3 times with 1X Wash Buffer. **Allow the Wash Buffer to sit in the wells for 10–15 seconds for each wash before aspiration**.

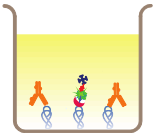
4 Add Substrate Solution



4.1. Add 100 µL Substrate Solution to each well. The substrate solution begins to turn blue.
4.2. Incubate for 15 minutes at room temperature.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution



Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and analyze results

1. Read the absorbance on a spectrophotometer using 450 nm as the primary wavelength (optionally 620 nm as the reference wavelength; 610 nm to 650 nm is acceptable as well) Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both the samples and the controls. Read the plate immediately after adding the Stop Solution.
2. Calculate Neutralization (%) for unknown samples:

   Neutralization (%) = [1 – (Absorbance of unknown sample/Absorbance of negative control)] × 100.
   - ≥20% = Positive
   - <20% = Negative

Performance characteristics

Reproducibility

Intra-assay precision

Reproducibility within the assay was evaluated in three independent experiments. Each assay was carried out with six replicates of serum samples containing different concentrations of SARS-CoV-2 neutralizing antibodies. Duplicates of Negative Control and Positive Control were run on each plate. The following data show the mean % Neutralization for SARS-CoV-2 antibodies and the coefficient of variation for each sample. The calculated overall intra-assay coefficient of variation was 1.1% (% Neutralization).

Sample	Experiment no.	Mean % Neutralization	% Coefficient of Variation
1	1	95	0.1
	2	95	0.1
	3	94	0.1
2	1	87	0.2
	2	87	0.5
	3	86	0.5
3	1	85	0.3
	2	86	0.5
	3	85	0.7
4	1	83	0.8
	2	83	0.6
	3	82	0.6
5	1	47	3.1
	2	52	2.9
	3	51	5.4

Inter-assay precision

Assay to assay reproducibility within one laboratory was evaluated in three independent experiments. Each assay was carried out with six replicates of serum samples containing different concentrations of SARS-CoV-2 neutralizing antibodies. Duplicates of Negative Control and Positive Control were run on each plate. Data below show the mean % Neutralization of SARS-CoV-2 antibodies and the coefficient of variation calculated on 18 determinations of each sample. The calculated overall inter-assay coefficient of variation was 1.4% (% Neutralization).

Sample	Mean % Neutralization	% Coefficient of Variation
1	95	0.5
2	86	0.4
3	85	0.4
4	83	0.7
5	50	5.2

Parallelism

Serum and plasma of SARS-CoV-2 positive samples with different levels of SARS-CoV-2 neutralizing antibodies and SARS-CoV-2 negative samples were analysed at 2-fold serial dilutions with two replicates each.

Sample	Dilution	Mean % Neutralization
SARS-CoV-2 positive sample 1	1:50	87
	1:100	81
	1:200	69
SARS-CoV-2 positive sample 2	1:50	85
	1:100	74
	1:200	59
SARS-CoV-2 positive sample 3	1:50	77
	1:100	67
	1:200	55
SARS-CoV-2 positive sample 4	1:50	62
	1:100	53
	1:200	44
SARS-CoV-2 negative sample 1	1:50	3
	1:100	1
	1:200	3
SARS-CoV-2 negative sample 2	1:50	1
	1:100	1
	1:200	1
SARS-CoV-2 negative sample 3	1:50	5
	1:100	–2
	1:200	2

Sample stability

Freeze-thaw stability

Serum samples were stored at –20°C and subjected to three freeze-thaw cycles, after which the % Neutralization of SARS-CoV-2 antibodies were determined. There was no significant loss in % Neutralization of SARS-CoV-2 antibodies detected by freezing and thawing.

Storage stability

Aliquots of serum samples were stored at –20°C, 2–8°C, or room temperature (RT) for 24 hours, after which the % Neutralization of SARS-CoV-2 antibodies was determined. There was no significant % Neutralization of SARS-CoV-2 antibodies detected during storage under the conditions that were tested.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0024968

Revision	Date	Description
B.0 (31)	26 August 2022	Correcting formula in "Read the plate and analyze results".
A.0 (30)	08 March 2021	New document for SARS-CoV-2 Neutralizing Antibody ELISA Kit.