Nirsevimab ELISA Kit

Cat #: orb2975673 (manual)

Materials Provided & Storage Conditions

ITEM	FORMAT	DESCRIPTION	STORAGE CONDITIONS
Pre-coated Microplate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) precoated with recombinant Human respiratory syncytial virus A (HRSV) Fusion glycoprotein F0.	Store in sealed at -20℃.
Nirsevimab Standard	2 bottles	3,000 ng/bottle of lyophilized Nirsevimab. Reconstitute in 1 mL Standard Diluent before used.	Store at -20℃.
Detection A	1 vial	60 µL/vial of HRP labeled Nirsevimab (including preservative), 1:100 diluted by Assay Diluent before used.	Store at -20℃.
Standard Diluent	1 bottle	25 mL/bottle diluent (including preservative) was used to dilute the Standard and Samples.	Store at 4℃.
Assay Diluent	1 bottle	25 mL/bottle diluent (including preservative) was used to dilute the Detection A.	Store at 4℃.
20 × Wash Buffer	1 bottle	25 mL/bottle of a 20-fold concentrated solution of buffered surfactant with preservative, 1:20 diluted by deionized water before used.	Store at 4℃.
Color Reagent	1 bottle	12 mL/ bottle of TMB (Tetramethylbenzidine).	Store at 4℃.
Stop Solution	1 bottle	6 mL/ bottle.	Store at 4℃.
Plate Sealers	4 strips	Adhesive strips.	Store at RT.

Intended Use

This kt is designed for the quantitative determination of Nirsevimab concentration in serum and plasma.

Principle of The Assay

This assay employs the quantitative competitive enzyme immunoassay technique. Recombinant Human respiratory syncytial virus A (HRSV) Fusion glycoprotein F0 has been pre-coated onto a microplate.

Standards or samples are premixed with HRP-labeled antibody and then pipetted into the wells. Nirsevimab in the sample competitively binds to the pre-coated protein with HRP-labeled Nirsevimab. After washing away any unbound substances, a substrate solution is added to the wells and color develops in inversely proportion to the amount of Nirsevimab bound in the initial step. The color development is stopped, and the intensity of the color is measured.

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Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 630 nm or 620 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- 500 mL graduated cylinder.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. Handle all blood and serum as if capable of transmitting infectious agents. The NCCLS provides recommendations for handling and storing serum and plasma specimens (Approved Standard-Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).

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℃. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ⩽ -20℃. Avoid repeated freeze-thaw cycles.

Reagent Preparation

Bring all reagents to room temperature before use.

20-fold Wash Buffer Concentrate - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 25 mL of Wash Buffer Concentrate to 475 mL of deionized or distilled water to prepare 500 mL of **Wash Buffer**.

Serum and Plasma - Serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 μL of sample + 990 μL of Standard Diluent (diluent 1:99). If the sample value is outside the range of the standard curve, the dilution can be adjusted appropriately, and the assay can be redetermined. If the antibody concentration in the sample can be estimated and the assay can be performed simultaneously by diluting several gradients prior to the experiment.

Standard - Reconstitute with 1 mL **Standard Diluent**, this reconstitution produces a **stock solution** of 3,000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 3,000 ng/mL is the first standard point, and the concentration of the 7 standard sample were 3,000 ng/mL, 1,500 ng/mL, 750 ng/mL, 375 ng/mL, 187.5 ng/mL, 93.75 ng/mL, 46.88 ng/mL respectively.

The appropriate **Standard Diluent** serves as the zero standard (0 ng/mL).

Detection A (working solution) - Shake and mix before using. Centrifuge instantaneously with palm centrifuge to make the liquid at the bottom of the tube. Dilute the **Detection A** 1: 100 times to the working concentration with **Assay Diluent**.

Assay Procedure

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

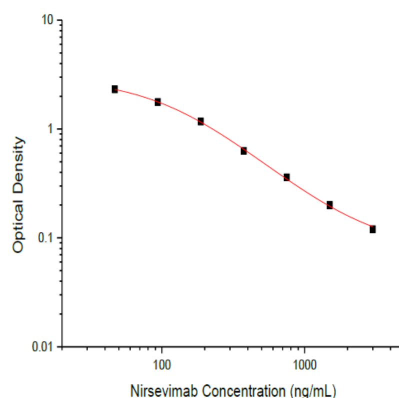
1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 100 µL premixed solution (50 µL diluted standard/sample + 50 µL Detection A working solution) to each well. Cover with the adhesive strip provided. Incubate for 1 hours at 37°C.
4. Aspirate each well and wash, repeating the process five times. Wash by filling each well with Wash Buffer (300 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **Color Reagent** to each well. Incubate for 15 minutes at 37°C. Protect from light.
6. Add 50 µL of **Stop Solution** to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

Determine the optical density of each well within 10 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 630 nm or 620 nm. If wavelength correction is not available, subtract readings at 630 nm or 620 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

Average the duplicate readings for each standard and sample. 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 through the points on the graph. The data may be linearized by plotting the log of the Nirsevimab concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.



Concentration (ng/mL)	OD1	OD2	Average
0	3.05	3.08	3.07
46.88	2.25	2.37	2.31
93.75	1.77	1.77	1.77
187.5	1.17	1.17	1.17
375	0.59	0.67	0.63
750	0.36	0.36	0.36
1500	0.19	0.20	0.20
3000	0.12	0.11	0.12

Range

46.88 - 3,000 ng/mL

Sensitivity

The minimum detectable dose (MDD) of Nirsevimab is typically less than 13.72 ng/mL.

The MDD was determined by subtracting two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

Precision

Intra-Assay Precision (Precision within an assay): <20%

Three samples of known concentration were tested sixteen times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays): <20%

Three samples of known concentration were tested in twenty four separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean (ng/mL)	1603.5	294.5	80.3	876.0	248.9	80.7
Standard deviation	305.2	11.6	4.6	167.3	22.1	10.1
CV (%)	19.0	3.9	5.7	19.1	8.9	12.5

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Stability

When the kit was stored at the recommended temperature for 6 months, the signal intensity decreased by less than 20%.

For unopened kits, if you want to prolong the storage time, please store the Standard, Detection A and Microplate at - 20 ℃, the rest reagents should be store at 4℃.

Recovery

The recovery of Nirsevimab spiked to three different levels in human serum samples throughout the range of the assay was evaluated.

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=4)	1:100	98	85-119
	1:200	97	86-106

Linearity

To assess the linearity of the assay, human serum samples were spiked with high concentrations of Nirsevimab and diluted with the Standard Diluent to produce samples with values within the dynamic range of the assay. (Human serum samples were prediluted 100-fold)

Sample	Dilution factor	Average (%)	Range (%)
Human serum (n=3)	1:02	97	93-105
	1:04	109	105-117
	1:08	113	108-117
	1:16	94	83-108

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Troubleshooting Guide

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After stopping the reaction, the entire plate shows a uniform yellow or light color, or the standard curve is linear but the background signal is excessively high.	Yellowing of the entire plate may be caused by incorrect addition of reagents.	Before starting the experiment, verify the components and lot numbers of all reagents to ensure they belong to the same kit. Reagents from different kits or different lot numbers must not be mixed.
	The ELISA plate was not washed sufficiently.	Ensure that the same volume of Wash Solution is added to each microwell during the washing process. After washing, firmly tap the ELISA plate on absorbent paper to remove any residual buffer.
	Incubation time was too long.	Strictly follow the procedures outlined in the manual.
	Streptavidin-HRP contaminated the pipette tip or TMB container, or the positive control contaminated the pre-coated microplate.	Replace pipette tips when dispensing different reagents. Use separate containers when preparing different reagent components, and always use a pipette during handling.
	The concentration of Biotinylated Antibody or Streptavidin-HRP was too high.	Verify that concentration calculations are correct, or perform further dilution if necessary.
	The substrate was exposed to light or contaminated prior to use.	Store reagents in the dark at all times prior to substrate addition.
	Color development time was too long.	Strictly follow the procedures outlined in the manual.
	An incorrect filter was used when reading the absorbance value.	When TMB is used as the substrate, measure absorbance at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are	Components from different reagent sets were mixed.	Carefully read reagent labels when preparing or using them.

colorless, and the positive control is not clearly detectable. s	During plate washing or sample/enzyme addition, the enzyme label was contaminated or inactivated, resulting in loss of its ability to catalyze color development.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), and ensure that the container used to prepare the Wash Solution has been thoroughly cleaned.
	A reagent or procedural step was omitted.	Review the manual carefully and strictly follow the operating procedures.

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard appears normal, but the sample color is weak.	The sample contains NaN_3 preservative, which inhibits the enzyme reaction.	Samples must not contain NaN_3.
	The sample being tested may not contain strongly positive material, so the result may be normal.	If there is any doubt about the results, repeat the assay.
The visual result appears normal, but the microplate reader values are low.	An incorrect filter was used for absorbance measurement.	When TMB is used as the substrate, absorbance should be measured at 450 nm.
All wells, including Standards and Samples, show weak color development.	Insufficient incubation time.	Ensure accurate timing using a timer.
	Inadequate color development.	Typically 15–30 minutes.
	Excessive washing, or the dilution ratio of the concentrated Wash Buffer does not meet requirements.	Minimize the impact of washing by diluting the concentrated Wash Buffer and setting the washing time according to the manual. Accurately record the number of washes and the volume used.
	Poor quality distilled water.	The prepared Wash Buffer should be tested to confirm that the pH is neutral.
	During plate washing or sample addition, the enzyme label was contaminated or inactivated, resulting in loss of catalytic activity.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3), that the container used to prepare the Wash Buffer has been thoroughly cleaned, and that the purified water used meets the required standards and is not contaminated.
	The kit has expired or was improperly stored.	Use the kit within its expiration date and store it according to the conditions recommended in the manual to avoid contamination.

	Reagents and samples were not equilibrated to room temperature before use.	Allow all reagents and samples to equilibrate at room temperature for approximately 30 minutes before use.
	Insufficient pipetting volume, overly rapid dispensing, excessive liquid remaining on the inner wall of the tip, or unclean tip walls.	Calibrate the pipette properly. Ensure tips are compatible and fit securely, pipette at an appropriate speed, and fully dispense the liquid. Tips should have clean inner walls and be used only once
Poor repeatability.	Incubation temperature was not properly controlled.	Maintain a constant incubation temperature and avoid localized temperature extremes.
	Excess liquid remained on the inner wall of the wells during liquid addition.	When adding liquids, dispense along the lower inner wall of the wells without touching the bottom.
	Reuse of consumables.	Replace pipette tips when drawing different reagents, and use separate containers when preparing different reagent components.
	The bottom of the microwell is scratched or contaminated.	Handle the plate carefully. Avoid touching the well bottoms and clean the bottom of the microplate to remove dirt or fingerprints.
		Perform technical replicates of the same sample three times, ensuring that at least two values are comparable.
	Cross-contamination during sample addition.	Minimize the risk of cross-contamination during sample addition.
The color development across the plate is uneven and irregular.	Cross-contamination during manual plate washing.	When washing plates manually, discard the first three washes immediately, then allow soaking during subsequent washes to reduce cross-contamination.
	Cross-contamination during plate tapping.	Use appropriate absorbent paper when tapping the plate. Avoid introducing foreign material into the wells, and avoid tapping in the same position repeatedly to reduce cross-contamination.

Description of results	Possible reason	Recommendations and precautions
The color development of the plate is uneven and irregular	The liquid dispensing head of the plate washer is clogged, leading to improper liquid dispensing or excessive residual liquid after aspiration, which results in uneven and irregular color development across the plate.	Unclog the liquid dispensing head to ensure that each well is properly filled with wash solution during plate washing and that minimal residual liquid remains after aspiration.

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	Incomplete centrifugation of the sample, resulting in coagulation within the reaction wells or interference from sediment or residual cellular components.	Serum and plasma samples should be fully centrifuged at 3000 rpm for more than 6 minutes.
	The sample was stored for an excessively long period, leading to contamination.	Samples should be kept fresh or stored at low temperatures to prevent contamination.
	Incorrect preparation of the Wash Solution or direct use of the concentrated Wash Solution.	Prepare all reagents strictly according to the manual.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.

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