Anti-HTNV N/Nucleoprotein hIgG Antibody ELISA Kit (KAV46601)

MATERIALS PROVIDED & STORAGE CONDITIONS

PART	FORMAT	DESCRIPTION	STORAGE CONDITIONS
Pre-coated Microplate	1 plate	96 well polystyrene microplate (12 strips of 8 wells) precoated with recombinant HTNV Nucleoprotein.	Store in sealed at -20 ℃.
Anti-HTNV Nucleoprotein hI gG Standard	2 bottles	20 ng/bottle of lyophilized Anti-HTNV Nucleoprotein hIgG. Reconstitute in 1 mL Standard Diluent before used.	Store at -20 ℃.
Detection A	1 vial	120 µL/vial of Biotin labeled Mouse Anti-Human IgG antibody (including preservative), 1:100 diluted by Assay Diluent before used.	Store at -20 ℃.
Detection B	1 vial	120 µL/vial of Streptavidin-HRP (including preservative), 1:100 diluted by Assay Diluent before used.	Store at -20 ℃.
Standard Diluent	2 bottles	25 mL/bottle diluent (including preservative) was used to dilute the Standard and Samples.	Store at 2 - 8 ℃.
Assay Diluent	1 bottle	25 mL/bottle diluent (including preservative) was used to dilute the Detection A and Detection B.	Store at 2 - 8 ℃.
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 2 - 8 ℃.
Color Reagent	1 bottle	12 mL/bottle of TMB (Tetramethylbenzidine).	Store at 2 - 8 ℃.
Stop Solution	1 bottle	6 mL/bottle.	Store at 2 - 8 ℃.
Plate Sealers	4 strips	Adhesive strips.	Store at RT.

* Provided this is within the expiration date of the kit.

Note：Unopened kits should be stored at 2 - 8 ℃ upon receiving.

For opened kits, if you want to prolong the storage time, please store the Standard, Detection A, Detection B and Microplate at - 20 ℃, the rest reagents should be store at 2 - 8 ℃.

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INTENDED USE

Used for the quantitative determination of Anti-HTNV Nucleoprotein hIgG concentration in serum and plasma.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative indirect enzyme immunoassay technique. Recombinant HTNV Nucleoprotein has been pre-coated onto a microplate. Standards or samples are pipetted into the wells and any Anti-HTNV Nucleoprotein hIgG present is bound by the immobilized protein. After washing away any unbound substances, a biotin-labeled Mouse Anti-Human IgG antibody is added to the wells. After washing away any unbound substances, Streptavidin-HRP is added to the wells. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Anti-HTNV Nucleoprotein hIgG bound in the initial step. The color development is stopped and the intensity of the color is measured.

OTHER SUPPLIES REQUIRED

• 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).

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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 1,000-fold dilution. A suggested 1,000-fold dilution can be achieved by adding 10 µL of sample to 90 µL of Standard Diluent. Then add 5 µL of the diluted sample to 495 µL Standard Diluent. 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 antigen 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 20 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. 20 ng/mL is the first standard point, and the concentration of the 7 standard sample were 20 ng/mL, 10 ng/mL, 5 ng/mL, 2.5 ng/mL, 1.25 ng/mL, 0.63 ng/mL, 0.31 ng/mL respectively. The appropriate **Standard Diluent** serves as the zero standard (0 ng/mL).

Note： Please use a new standard for each assay and discard after use. Avoid freeze-thaw cycle.

Detection A (working solution) - Shake and mix before used. 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**.

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

Note： Please perform simple centrifugation to collect Detection A and Detection B before use. Prepare Detection A and Detection B immediately before use and avoid diluting the entire stock at once.

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 standard or samples to each well. Cover with the adhesive strip provided. Incubate for 1 hour at 37 ℃.

4. Aspirate each well and wash, repeating the process three 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 **Detection A** (working solution) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37 ℃.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of **Detection B** (working solution) to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37 ℃.

8. 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.

9. Add 100 µL of **Color Reagent** to each well. Incubate for 15 minutes at 37 ℃. Protect from light.

10. 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.

11. 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.

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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 Anti-HTNV Nucleoprotein hIgG 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.

ASSAY RANGE

0.31 - 20 ng/mL

SENSITIVITY

The minimum detectable dose (MDD) of Anti-HTNV Nucleoprotein hIgG is typically less than 0.28 ng/mL.

The MDD was determined by adding 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): <10%

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

Inter-Assay Precision (Precision between assays): <15%

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

STABILITY

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

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TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Bubbles in the wells	Tap plate gently to disperse bubbles
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
High background	Plate is not washed properly	Make sure the wash apparatus works properly
	Incorrect incubation times and/or temperatures	The OD value increased gradually along with the time. Reduce the color developing time properly
Weak/No Signal	Pipetting errors	Make sure the pipette is calibrated
	The working solution not be prepared immediately before use	The working solution should be prepared immediately before use and should not be stored
	Volumes errors	Repeat assay with the required volumes in manual
	The plate is not incubated for proper time or temperature	Follow the manual to repeat assay
	Detection A working solution is not completely mixed with the samples	After adding the Detection A into the wells, make sure the detection A and the samples are mixed thoroughly

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