Human NUP210 Antibody ELISA Kit

Cat. No.:DEIA1226
Pkg.Size:96T

Intended use

The Human NUP210 Antibody ELISA kit is designed to detect and quantify the level of human NUP210 Antibody in cell culture supernatant, serum, plasma and tissue.

General Description

The nuclear pore complex is a massive structure that extends across the nuclear envelope, forming a gateway that regulates the flow of macromolecules between the nucleus and the cytoplasm. Nucleoporins are the main components of the nuclear pore complex in eukaryotic cells. The protein encoded by this gene is a membrane-spanning glycoprotein that is a major component of the nuclear pore complex.

Principle Of The Test

The qualitative immunoenzymatic determination of antibodies against NUP210 antigen is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with NUP210 antigen to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, and goat anti-human IgG antibodies (anti-IgG) conjugated to horseradish peroxidase (HRP) are added. This conjugate binds to antigen-antibody complexes. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of NUP210 antigen -specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Reagents And Materials Provided

Human NUP210 Ag Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
Low Avidity Control,1 mL per bottle: 1 bottle
Positive control,1 mL per bottle: 1 bottle
Negative control,1 mL per bottle: 1 bottle
HRP-Conjugate Reagent, 15 ml per bottle with preservatives: 1 bottle
Assay Solution, 80 mL per bottle with preservatives: 1 bottle
Wash Solution Concentrate (10×), 80 mL with preservatives: 1 bottle
Chromogen Solution A, 10 mL per bottle: 1 bottle
Chromogen Solution B, 10 mL per bottle: 1 bottle
Stop Solution, 10 mL per bottle: 1 bottle

Materials Required But Not Supplied

1. A standard ELISA plate reader for absorbance at 450 nm.
2. Calibrated adjustable precision pipettes (single channel and multi channel), preferably with disposable plastic tips.
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software or graph paper.
6. Polypropylene tubes.

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
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7. Graduated cylinders and calibrated beakers in various sizes.

Storage

Store all reagents at 2 to 8℃.

Specimen Collection And Handling

1. Sample Collection: Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC and the volume should >50 µl.
2. Storage: Store samples at 2-8 ℃. Samples not required for assaying within 3 days should be stored frozen (-20℃ or lower).Multiple freeze-thaw cycles should be avoided.

Reagent Preparation

1. Allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes and mix gently.
2. Wash Solution: Make a 1:10 dilution of Wash Solution Concentrate (10X) with deionized or distilled water in a clean plastic tube as needed. Label as Wash Solution. Store both the concentrate and the Wash Solution in the refrigerator. The diluted buffer should be used within one week.
3. Diluting Sample: Dilute each sample 1:101 with sample diluent(Add 5 µl serum into 500 µl Assay solution). The diluted buffer should be used within 6 hours.

Assay Steps

1. Determine the number of strips needed for the assay and remove excess microplate strips from the plate frame, return them to the foil pouch, and reseal.
2. Adding Sample: Add 100µl of samples and 100µl Low Avidity Control, Positive and Negative controls into their respective wells. Seal the plate with Plate Covers and incubate at 37℃ for 30min. Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Controls as to avoid cross-contamination.
3. Aspirate each well and wash 4 times. Wash by filling each well with Wash Solution (300 µL) using a multi-channel pipette, squirt bottle, manifold dispenser or autowasher. Allow the Wash Solution to soak for about 10-20 seconds before aspiration. After the last wash step, the plate is inverted and tapped dry on absorbent pad or paper towel. A thorough washing of the plate is extremely important to reduce background. (Prepare Wash Solution according to REAGENT PREPARATION.)
4. Add 100 µL of Streptavidin-HRP Solution to each well. Cover with a new adhesive strip. Incubate for 30 minutes at 37℃.
5. Aspirate each well and wash according to Step 3.
6. Dispense 50µl of Chromogen A and 50µl Chromogen B solution into each well including the Blank and mix by tapping the plate gently. Incubate the plate at 37℃ for 15 minutes avoiding light. The enzymatic reaction between the Chromogen A/B solutions produces blue color in Positive control and SSA antibody Positive sample wells.
7. Stopping Reaction: Using a multichannel pipette or manually, add 50µl Stop solution into each well and mix gently. Intensive yellow color develops in Positive control and SSA antibody positive sample wells.
8. Measuring the Absorbance: Calibrate the plate reader with the Blank well and read the absorbance at 450nm. If a dual filter instrument is used, set the reference wavelength at 630nm. Calculate the Cut-off value and evaluate the results. (Note: read the absorbance within 5 minutes after stopping the reaction)

Quality Control

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the sample being analyzed.
1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 at 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm or at 450nm after blanking.

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3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

Interpretation of Results

sample/control=O.D. with sample/O.D. with Low Avidity Control
Negative Results (sample/control<1) : samples giving absorbance less than the O.D. with Low Avidity Control are negative for this assay, which indicates that no antibodies to NUP210 antigen have been detected with this anti-NUP210 antibody ELISA kit. Therefore, there are no serological evidences for resent infections with NUP210 and the patient is probably not infected with the virus.
Positive Results (sample/control≥1) : samples giving an absorbance greater than, or equal to the O.D. with Low Avidity Control are initially reactive, which indicates that antibodies to NUP210 antigen have probably been detected with this anti-NUP210 antibody ELISA kit. Any reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for anti-NUP210 antibody. Positive results with anti-NUP210 antibody detection indicate possible recent infection with NUP210.
Borderline (sample/control =0.9-1.1): Samples with absorbance to O.D. with Low Avidity Control between 0.9 and 1.1 are considered borderline samples and retesting of these samples in duplicates is recommended. Repeatedly positive samples can be considered positive for anti-NUP210 antibody.
The result from this assay should not be used alone to establish the infection state.

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 29.0%.

Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is≥99.0%.

Reproducibility

Intra-Assay: CV≤15%
Inter-Assay: CV≤15%

Precautions

1. The Stop solution (2M H2SO4) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. Proclin 300 used as a preservative can cause sensation of the skin.
2. All specimens from human origin should be considered as potentially infectious. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
3. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
4. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.

Limitations

This is a qualitative assay and the results cannot be use to measure antibodies concentrations.

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