Human CCP Antibody ELISA Kit

Cat. No.:DEIA1227
Pkg.Size:96T

Intended use

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

General Description

Anti-citrullinated protein antibodies (ACPA) or anti-cyclic citrullinated protein antibodies (anti-CCP) are autoantibodies (antibodies directed against one or more of an individual's own proteins) that are frequently detected in the blood of rheumatoid arthritis patients. The main epitope for these antibodies is filaggrin,and there is cross-reactivity between ACPA and anti-keratin and anti-perinuclear factor.During inflammation, arginine residues in proteins such as vimentin can be enzymatically converted into citrulline ones (a process called citrullination), and, if their shapes are significantly altered, the proteins may be seen as antigens by the immune system, thereby generating an immune response. ACPAs have proved to be powerful biomarkers that allow the diagnosis of rheumatoid arthritis (RA) to be made at a very early stage.

Principle Of The Test

The quantitative immunoenzymatic determination of antibodies against CCP antigen is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microtiter strip wells are precoated with CCP 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 CCP 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 CCP Ag Coated Wells, 96-well polystyrene microplate (12 strips of 8 wells): 1 plate
Standard 1(1600 RU/ml),1 mL per bottle: 1 bottle
Standard 2(400 RU/ml),1 mL per bottle: 1 bottle
Standard 3(200 RU/ml),1 mL per bottle: 1 bottle
Standard 4(50 RU/ml),1 mL per bottle: 1 bottle
Standard 5(25 RU/ml),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, 60 mL per bottle with preservatives: 1 bottle
Wash Solution Concentrate (10×), 60 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

45-16 Ramsey Road Shirley, NY 11967, USA
Tel: 631-624-4882 ·Fax:631-614-7828
E-mail: info@creative-diagnostics.com
www.creative-diagnostics.com

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.
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 1:50 with sample diluent. 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:
Quantitive test:Add 100µl of samples and 100µl Standard, Positive and Negative controls into their respective wells.
Qualitive test: Add 100µl of samples and 100µl Standard 5, 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

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

Calculation

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.
Manual Plotting: Plot on graph paper the absorbance of the standards against the standard concentration. Known concentrations of Human CCP are plotted on the X-axis and the corresponding absorbances on the Y-axis. The standard curve should result in a straight line that shows a direct relationship between Human CCP concentrations and the corresponding absorbances. The concentration of Human CCP in samples may be determined by plotting the sample absorbances on the Y-axis, then drawing a horizontal line to intersect with the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Human CCP concentration.
Note: Samples producing signals greater than that of the highest standard should be diluted in Assay Solution and reanalyzed. Multiply the measured concentration by the appropriate dilution factor.
Plate Reader: An alternative approach is to use an ELISA curve fitting software. A linear curve plot is expected to produce the best fit of the resulting sample concentrations.

Interpretation of Results

1. Quality
sample/control=O.D. with sample/O.D. with Standard 5
Negative Results (sample/control<1) : samples giving absorbance less than the O.D. with Standard 5 are negative for this assay, which indicates that no antibodies to CCP antigen have been detected with this anti-CCP antibody ELISA kit. Therefore, there are no serological evidences for resent infections with CCP 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 Standard 5 are initially reactive, which indicates that antibodies to CCP antigen have probably been detected with this anti-CCP antibody ELISA kit. Any reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for anti-CCP antibody. Positive results with anti-CCP antibody detection indicate possible recent infection with CCP.
Borderline (sample/control =0.9-1.1): Samples with absorbance to O.D. with Standard 5 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-CCP antibody.
The result from this CCP should not be used alone to establish the infection state.
2. Quantify
Sample with concentration < 25 RU/ml are negative for this assay.
Sample with concentration≥25 RU/ml are positive for this assay.
Sample with concentration=25 RU/ml-27 RU/ml are considered borderline samples and retesting of these samples in duplicates is recommended. Repeatedly positive samples can be considered positive for anti-CCP antibody.

Sensitivity

The sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 82%.

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Specificity

The specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is≥95%.

Reproducibility

Intra-Assay: CV≤20%
Inter-Assay: CV≤20%

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.

REFERENCES

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