NOVATEINBIO
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Anti-CFG antibody ELISA Kit

(Cat# BG-HUM10021)

Important notes

Before using this product, please read this manual carefully; After reading the subsequent contents of this manual, please note the following specially :

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommended that all standard, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over-develop)
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB substrate is light-sensitive. Avoid prolonged exposure to the light.

Anti-CFG ELISA kit is an ELISA test system for the quantitative measurement of IgG class autoantibodies against citrullinated Fibrinogen (CFG) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Assay principle

- Highly purified citrullinated Fibrinogen (CFG) is bound to micro-wells.
- The determination is based on an indirect enzyme linked immune reaction with the following steps: Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and un-specifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue color product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photo-metrically at 450 nm.

Background

- Rheumatoid arthritis (RA) is one of the most common autoimmune diseases. It is characterized by a progressive inflammation of the joints, leading to gradual damage and loss of their function. Early diagnosis of RA and immediate onset of an appropriate

treatment is essential for prevention of complete joint damage. In addition to rheumatoid factors, autoantibodies against citrullinated Fibrinogen (ACFG) could be valuable tools for the serological diagnosis of early RA.

- It has been demonstrated in numerous studies that antibodies against citrullinated peptides from enolase, fibrinogen and especially vimentin occur in RF-negative patients. Citrullinated vimentin has been detected in the rheumatoid synovial tissue of RA patients and is involved in the initiation of ACPA production.
- Autoantibodies against mutated citrullinated Fibrinogen (Anti-CFG) are sensitive and specific markers for RA. They correlate with an erosive course of disease with severe joint damage and extra-articular manifestations. A strong correlation between Anti-CFG titers in RA patients and disease activity score (DAS) has been described.
- Anti-CFG ELISA could detects autoantibodies very early– sometimes even years before symptoms become evident. Persons without symptoms but with an increased Anti-CFG antibody titer could be at high risk for future RA development. Furthermore, a positive result is predictive for a severe course of RA.

Material provided

1. 1 x 96-well microplate, coated with CFG antigens.
2. Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL
3. Calibrator B 2 U/ml, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL
4. Calibrator C 4 U/ml, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL.
5. Calibrator D 10 U/ml, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL
6. Calibrator E 30 U/ml, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL
7. Calibrator F 100 U/ml, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. 1 x 1.5 mL
8. Control positive, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis. 1 x 1.5 mL
9. Control negative, containing CFG antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis. 1 x 1.5 mL
10. Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x). 20 mL
11. Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use. 15 mL
12. Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc. 20 mL
13. Stop solution; contains acid. Ready to use.
14. Instruction for Use.
15. Certificate of Analysis

Material required

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device

- Distilled or deionized water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

Specimen collection, storage and handling

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

Storage and stability

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the un-opened test kit is 18 months from day of production.

  Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Procedural notes

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, perform the test without interruption.
- Double determinations may be done. By this means pipetting errors may become

obvious.

- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

Warning and precautions

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit

reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

Reagent preparation

Wash Buffer

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a volume of 1000 ml prior to use.

Sample Buffer P

Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionized water to a final volume of 100 ml.

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

Assay procedures

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette **100 µl** of calibrators, controls and prediluted patient samples into the wells. Incubate for **30 minutes** at room temperature (20-28 °C).

   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.

2. Dispense **100 µ**l of enzyme conjugate into each well. Incubate for **15 minutes** at room temperature.

   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.

3. Dispense **100 µ**l of TMB substrate solution into each well. Incubate for **15 minutes** at room temperature

4. **Add 100 µl** of stop solution to each well of the modules Incubate for **5 minutes** at room temperature.

Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed color is stable for at least 30 minutes. Read during this time.

Validation

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit.

If these quality control criteria are not met the assay run is invalid and should be repeated.

Result calculation

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation.

Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance evaluation

Calibration

This assay system is calibrated in relative arbitrary units. It is calibrated against an external anti-CFG Assay, since no international reference sera for RA diagnostic are available so far.

Measuring range

The calculation range of this ELISA assay is

0 - 100 U/ml

Expected value

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 2 U/ml

Interpretation of results

Negative: < 2 U/ml

Positive: >= 2 U/ml

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity

for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

94 to 101%

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample .	Mean U/ml	. CV %
1	8.6	8.8
2	33.8	6.5
3	97.5	7.6

Inter-Assay		
Sample .	Mean U/ml	. CV %
1	26.4	9.6
2	75.9	9.9
3	34.7	8.6

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