

**User's Manual**

ANCA-C (PR3) ELISA Kit

REF DEIA3141 Σ 96T**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The ANCA-C (PR3) ELISA Kit is a test system for the quantitative measurement of IgG class autoantibodies against proteinase 3 (PR3) in human serum or plasma.

General Description

Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides. Anti-PR3 characterizes granulomatosis with polyangiitis (GPA, formerly: Wegener's granulomatosis). The test supports the differential diagnosis of vasculitis when used in combination with other laboratory and clinical findings.

Principles of Testing

Highly purified Proteinase 3 (PR3) is bound to microwells. Antibodies against the coated antigen, if present in diluted sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman antibodies immunologically detect the bound sample antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

Reagents And Materials Provided

1. Divisible microplate: Consisting of 12 modules of 8 wells each. Ready to use. 96 (8x12) wells
2. Calibrators A-F (0, 5, 10, 20, 40, 100 U/mL), containing serum/buffer matrix (PBS, BSA, detergent, NaN₃ 0.09%), yellow. Ready to use. 1.5 mL x 6
3. Control positive (1) and negative (2), containing PR3 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. 1.5 mL x 2
4. Sample buffer, containing PBS, BSA, detergent, preservative NaN₃ 0.09%, yellow, 5x conc. 20 mL
5. Enzyme conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use. 15 mL
6. TMB substrate; containing 3,3',5,5'-Tetramethylbenzidin, colorless. Ready to use. 15 mL
7. Stop solution; contains acid. Ready to use. 15 mL
8. Wash Buffer, containing Tris, detergent, preservative NaN₃ 0.09%; 50x conc. 20 mL

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm.

2. Data reduction software
3. Multi-Channel Dispenser or repeatable pipette for 100 μ L
4. Vortex mixer
5. Pipettes for 10 μ L, 100 μ L and 1000 μ L
6. Laboratory timing device
7. Distilled or deionized water
8. Measuring cylinder for 1000 mL and 100 mL
9. Plastic container for storage of the wash solution

Storage

Store the kit at 2-8°C in the dark. Do not expose test reagents to heat, sun or strong light during storage and usage. Store microplate sealed and desiccated in the clip bag provided. Unopened reagents are stable until expiration of the kit. See labels for individual batch. Diluted Wash solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipema should be avoided, but does not interfere with this assay.
4. Specimens should be stored at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.
7. Dilute the samples 1:100 before the assay: Put 990 μ L of prediluted sample buffer in a polystyrene tube and add 10 μ L of sample. Mix well. Note: Calibrators/Controls are ready to use and need not be diluted.

Reagent Preparation

Wash solution

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

Sample buffer

Sample buffer P: Prior to use dilute the contents (20 mL) of one vial of sample buffer 5 \times concentrate with distilled or deionized water to a final volume of 100 mL.

Assay Procedure

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

1. Pipet 100 μ L of calibrators, controls and prediluted samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28°C).
3. Discard the contents of the microwells and wash 3 times with 300 μ L of wash solution.
4. Dispense 100 μ L of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 μ L of wash solution.
7. Dispense 100 μ L of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature.
9. Add 100 μ L of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Quality Control

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.

Calculation

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of 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.

Interpretation Of Results

Negative: < 5 U/mL

Positive: \geq 5 U/mL

Performance Characteristics

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 5 U/mL

Detection Range

The calculation range of this ELISA assay is 0-100 U/mL

Detection Limit

Functional sensitivity was determined to be: 0.5 U/mL

Linearity

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.

Sample	Dilution	Observed U/mL	Expected U/mL	O/E [%]
1	1:100	78.9	78.9	100
	1:200	39.8	39.5	101
	1:400	20.6	19.7	105
	1:800	10.6	9.9	107
	1:1600	5.3	4.9	108
2	1:100	77.5	77.5	100
	1:200	37.4	38.8	96
	1:400	19.1	19.4	98
	1:800	9.7	9.7	100
	1:1600	5.0	4.8	104

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 run. Results for run-to-run precision are shown in the table below.

Intra-Assay			Inter-Assay		
Sample	Mean [U/mL]	CV [%]	Sample	Mean [U/mL]	CV [%]
1	10.9	4.7	1	10.4	6.2
2	24.6	2.8	2	23.4	8.8
3	58.5	2.8	3	60.7	3.9

Interferences

No interference has been observed with haemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. All reagents of this kit are strictly intended for research use only.
2. 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.
3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
5. Stop Solution contains acid, classification is non-hazardous. Avoid contact with skin.
6. Controls, Calibrators, Sample Buffer and Wash Solution contain Sodium Azide (NaN₃) 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
8. 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.
9. 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.
10. Exposure controls/personal protection: Wear protective gloves of nitrile rubber or natural latex.
11. Where protective glasses. Used according to intended use no dangerous reactions known.
12. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
13. For disposal of laboratory waste the national or regional legislation has to be observed.
14. Do not use kit components beyond their expiration dates.
15. Do not interchange kit components from different lots and products.
16. All materials must be at room temperature (20-28°C) prior to use.
17. Prepare all reagents and samples. Once started, perform the test without interruption.
18. Double determinations may be done. By this means pipetting errors may become obvious.
19. Perform the assay steps only in the order indicated.
20. Always use fresh sample dilutions.
21. Pipette all reagents and samples into the bottom of the wells.
22. To avoid carryover contamination, change the tip between samples and different kit controls.
23. Wash microwells thoroughly and remove the last droplets of Wash Solution.
24. All incubation steps must be accurately timed.
25. Do not re-use microplate wells.

Limitations

The abnormal and normal reference ranges for antibodies in samples should be regarded as

recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.