Human ANCA-P (MPO) ELISA Kit

Enzyme Immunoassay for the quantification of IgG antibodies against Myeloperoxidase in serum or plasma.

Catalog number: ARG80361

For research use only. Not for use in diagnostic procedures.

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A highly purified Myeloperoxidase (MPO) antigen has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any MPO antibodies (Ab) present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated anti-human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of MPO Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Concentration indicated on vial label)	6 vials (Ready-to-use) (0,5,10,20,40,100 U/ml)	4°C.
Controls	2 vials (Ready-to-use)	4°C

5X Sample buffer	20ml	4°C
HRP-Antibody conjugate	15ml (Ready-to-use)	4°C
50X Wash buffer	20ml	4°C
TMB substrate	15ml	4°C (Protect from light)
STOP solution	15ml	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. 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 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 50X Wash buffer into distilled water to yield 1X Wash buffer.
- 1X Sample buffer: Dilute 5X Sample buffer with distilled water before use.
- Patient sample: Dilute patient sample 1:100 with 1X sample buffer before assay, mix well. (e.g. 10 µl of sample + 990 µl of 1X sample buffer)

  Note: the controls and calibrators are ready-to-use and need not further dilution.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100µl of standards, controls, samples and zero controls into wells.
3. Incubate for 30 minutes at RT.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 100µl 1X Antibody solution into each well. Incubate for 15 minutes at RT.
6. Wash as according to step 4.
7. Add 100µl of TMB Reagent to each well. Incubate for 15 minutes at room temperature.
8. Add 100µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Negative: < 5 U/ml

Positive: > 5 U/ml

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of ACPA ranged from 5-100 U/ml. The mean MDD was 0.5 U/ml.

Specificity

No interference has been found with hemolytic or lipemic sera, or bilirubin-containing sera, or anticoagulants (Citrate, EDTA, Heparin)

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 4.5% and inter-assay precision was 5.4%.