ELISA kits available from ADI (see details at the web site)

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thryroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrcyte protein (MOG35-55) Ig's ELISA kit,

Instruction Manual No. M-3300-265-MPG

Human ANCA (Anti-MPO) IgG ELISA kit

Cat. # 3300-265-MPG, 96 tests

For quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma

For In Vitro Research Use Only

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Human ANCA (Anti-MPO) IgG ELISA KIT # 3300-265-MPG;
Kit Contents (96 tests):

Components	Cat. No.
Purified MPO antigen coated microwell strips (96 wells)	3300266P
Anti-MPO IgG **Stds. A-F** in a buffer (0, 5, 10, 20, 40, and 100 U/ml) 6 vials x 1.5 ml each	3300267A-F
Anti-MPO IgG **Negative Control** (1.5 ml each)	3300268N
Anti-MPO IgG **Positive Control** (1.5 ml each)	3300268P
Sample Diluent (5X), 20 ml (yellow color)	3300265-SD
Wash buffer (50X), 20 ml	3300265-WB
Anti-hIgG **HRP Conjugate**, 15 ml (light pink)	3300269
HRP **Substrate Solution** (TMB) , 15 ml	3300265-TM
Stop Solution, 15 ml	3300265-SS
Complete Instruction Manual, M-3300-265-MPG	1

Intended Use:

This kit measures human IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. **For in vitro research use only (RUO).**

Introduction:

Human ANCA (Anti-MPO) IgG ELISA kit is a for the quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides.

Anti-MPO differentiates microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) The test supports differential diagnosis of vasculitis, when used in conjunction with other clinical and laboratory findings.

Intra-Assay

Intra-Assay		
Sample	**Mean (U/ml)**	**CV (%)**
1	7.5	6.4
2	30.2	4.1
3	59.9	3.1

Inter-Assay		
Sample	**Mean (U/ml)**	**CV (%)**
1	7.0	5.0
2	33.8	4.9
3	78.3	6.3

Limit of detection:

The functional sensitivity was determined to be 0.5 U/ml.

Calibration

Since no international reference preparation for anti-MPO IgG is available, the assay system is calibrated in relative arbitrary units.

Measuring range:

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

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

Interpretation of results

Negative: < 5 U/ml, Positive: ≥ 5 U/ml

LIMITATIONS OF PROCEDURE

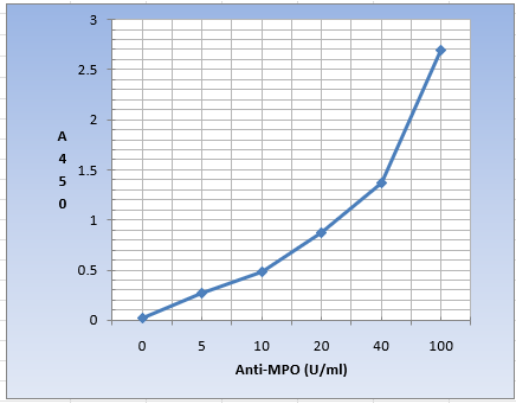
The Anti-MPO IgG ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

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

Typical Values of the standards (**do not use this for calculations**). These values are also lot specific and the following is only for demonstration purpose.

	Stds/samples	Mean OD450	Calculated Conc U/ml
A1/A2	0 U/ml	0.019	
B1/B2	5 U/ml	0.266	
C1/C2	10 U/ml	0.483	
D1/D2	20 U/ml	0.871	
E1/E2	40 U/ml	1.375	
F1/F2	100 U/ml	2.690	



Automation

The Anti-MPO IgG ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

Quality Control

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

PRINCIPLE OF THE TEST

Highly purified myeloperoxidase (MPO) is bound to microwells 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 unspecifically 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 colored 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 photometrically at 450 nm.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed , these could be stored at -20^{o}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (**20 ml stock in total of 1-liter**). **store at 4°C**.

Sample buffer (1:5) with distilled water (**20 ml stock in total of 100-ml**). **s**tore at 4°C for 30-day or until the expiration date printed on the label.

REAGENT PREPARATION FOR THE ASSAY

1. Dilute wash buffer 1:50 (20 ml stock in 980 ml water) and store at 4°C.
2. Dilute sample diluent 1:5 (20 ml stock in 80-ml water) and store at 4°C.
3. Dilute all samples to be tested 1:100 with sample diluent (10 ul sample in 990 ul of diluent or 5 ul sample in 495 ul diluent).
4. Bring all reagents and samples to room temperature (25-30°C)

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions.

Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE). .*

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute** samples (1:100) in 1x sample buffer. **Controls & standards provided in the kit are ready-to-use.** Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter).

2. Pipet **100 ul of sample** buffer (for use as blanks), negative, positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently for 5-10 seconds, cover the plate and incubate for **30 minutes** at room temp (20-28°C).

3. Aspirate and **wash the wells 3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add **100 ul of enzyme conjugate** into each well**.** Mix gently for 5-10 seconds. Cover the plate and incubate for **15 minutes** at room temp.

5. Aspirate and wash the wells **3 times** as above.

6. Dispense **100 ul TMB substrate per well**. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for **15 minutes**. **Blue color** develops in positive wells.

7. Stop the reaction by adding **100 ul** of stopping solution to all wells at the same timed intervals . Mix gently for 5-10 seconds. incubate for **5 minutes** at room temp **Blue color urns yellow**. Measure the absorbance of yellow color at 450 nm using an ELISA reader.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

QUALITY CONTROL

This test is only valid if the optical density at 450 nm for positive control (1) and negative control (2) as well as for the calibrator A and F complies with the respective range indicated on each vial If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Study results:

Study population	**n**	**n Pos %**
Crescendic glomerulenophritis	55	53 96.4
Morbus Wegener (cANCA pos)	20 1	5.0
Non-ANCA kidney disease	10 1	10.0
Normal human sera	120 3	2.5

Sensitivity: 98.2 %

Specificity: 96.7 %

Overall agreement: 97.1 %

Calculation of results

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

Multiply the sample values only if the samples were diluted more than 1:100 (e.g., if sample diluted by 1:500 then multiply the values by 5).

Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.