EIASON® p-ANCA (MPO)

IVD

Enzyme Immunoassay for the quantitative determination of IgG autoantibodies to myeloperoxidase in human serum or plasma

Kit instruction

For in-vitro use only

Product of

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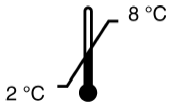
REF E07-009-96

2 °C 8 °C

CE

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	MPL	Microplate
REF	Order number	CONJ	Conjugate
	Product of	WASH	Wash Buffer Concentrate
	Storage	LOT	Batch code
CE	European Conformity	SUB	Substrate
	Expiry date	STOP	Stop Solution
DIL	Sample Diluent	PC NC	Calibrator Control Negative Control
CAL 0 - 5	Calibrators		

Intended use

For in-vitro use only.

EIASON® pANCA is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of certain autoimmune vasculitides such as microscopic polyarteritis and crescentic glomerulonephritis.

Summary

Anti-neutrophil cytoplasmic antibodies (ANCA) represent a group of autoantibodies directed towards cytoplasmic components of the neutrophil granulocytes and

monocytes. The classical methods for the determination of ANCA are immunofluorescence tests. With these indirect immunofluorescence (IF) techniques two main patterns are distinguished: a cytoplasmic (cANCA) and a perinuclear (pANCA) type.
The target antigen for 80-90 % of cANCA is proteinase 3 (PR3), a serine proteinase present in primary granules; 10-20 % of cANCA are directed to other proteins, such as bactericidal permeability-increasing protein (BPI). In rare cases, antibodies to elastase (4 %), lysozym (2 %) or cathepsin G (2 %) may show a cANCA-pattern. cANCA have also been detected in different non-rheumatic diseases.
Approximately 90 % of pANCA positive sera contain autoantibodies directed to myeloperoxidase (MPO), which is located in the granules of neutrophil granulocytes. Antibodies to other antigens e.g. Lactoferrin, Elastase, Cathepsin-G and also Lysozyme often result in a similar pANCA pattern. These atypical pANCA occur in collagenosis and related inflammatory rheumatic diseases. Besides, different untypical variants of pANCA IF patterns − granulocyte specific antinuclear antibodies (GS-ANA) − are indistinguishable from pANCA.
Therefore, a distinct interpretation and classification of the IF patterns is difficult and every positive IF-ANCA finding should be differentiated by ELISA techniques using the purified single antigens.
PR3 and MPO are well defined and reliable serologic markers for a definite group of primary systemic vasculitides (PSV), they are also called ANCA-associated vasculitides (AAV). The incidence is 1 in 1000 in the whole population and nearly 5 in 1000 in the elderly. The clinical appearance of AAV is characterised by manifestations in the kidneys, the lung and the respiratory tract.
PR3 is the most frequent component of cANCA and the landmark autoantigen in granulomatosis with polyangiitis (GPA, formerly named Wegener's granulomatosis) with a clinical specificity of more than 95% for the disease.
MPO, the main target antigen of pANCA, is present in 70% of patients with microscopic polyangiitis (MPA) and differentiates MPA from other autoimmune diseases.
Anti-PR3 and anti-MPO levels correlate with the clinical status; they are high in active disease. Antibody titres decrease under therapy and become undetectable after remission.
A survey of documented clinical indications, the corresponding immunofluorescence patterns and target antigens is given in the following table:

Diseases	IFA patterns	Target antigen
Systemic Vasculitic Syndromes		
Wegener´s Granulomatosis	c-ANCA, rare p-ANCA	PR3, rare MPO
Microscopic Polyangiitis	c-ANCA, p-ANCA	PR3, MPO
Churg-Strauss-Syndrome	p-ANCA	MPO
Polyarthritis nodosa	rare ANCA	Rare PR3 and MPO
Unclassified Vasculitis	rare	No PR3 and MPO
Collagen Diseases and other Rheumatic Disorders		
Rheumatoid arthritis	GS-ANA, p-ANCA, atypical ANCA	unknown, ANA, rare MPO, Lactoferrin
SLE	p-ANCA	rare MPO, Lactoferrin
Other Diseases		
Ulcerative Colitis		Cathepsin G, Lactoferrin

Assay principle

Highly purified myeloperoxidase (MPO) is bound to microwells. Antibodies against this antigen, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects the bound patient 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 color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm.
The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

Warnings and precautions

The EIASON® p-ANCA kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided. CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent. Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material. Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Damaged test kit

In case of serious damage to the test kit or components, the company IASON must be notified in writing at least one week after receiving the kit. Severely damaged single components should not be used for the test run.

Shelf life and storage of reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C. Do not use reagents beyond this date.
Opened reagents must be stored at 2-8°C. Microtiter wells must be stored at 2-8°C. After opening, keep any unused wells in the original foil packet (reseal with adhesive tape) and in the self-seal plastic bag with the desiccant provided.
Diluted WASH and DIL are stable for at least 30 days when stored at 2 - 8 °C. We recommend consumption on the same day.

Storage and preparation of serum samples

- 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 °C - 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.

Materials provided

Qty.1	MPL Divisible microtiter strips: 96 wells coated with highly purified myeloperoxidase (MPO). Ready to use.
6 vials, 1.5 mL each	CAL 0-5 Calibrators with IgG class Anti-MPO antibodies in a serum/buffer matrix (PBS, BSA, NaN3 <0.1% w/w), yellow; ready to use. Concentrations: 0; 5; 10; 20; 40; and 100 IAU/mL. Ready to use.
2 vials, 1.5 mL each	NC PC Negative and positive controls containing MPO antibodies in a serum/buffer matrix (PBS, BSA, NaN3 <0,1% (w/w), for the respective concentrations see the enclosed QC insert. Ready to use.
1 vial, 20 mL	DIL Sample buffer (PBS, BSA, NaN_3 <0.1% w/w), yellow, concentrate (5x). Dilute the contents of DIL with distilled or deionized water to a final volume of 100 mL prior to use.
1 vial, 15 mL	CONJ Enzyme conjugate solution containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. (PBS, Proclin 300 <0.5% (v/v), (light red). Ready to use.
1 vial, 15 mL	SUB TMB substrate solution, colourless. Ready to use.
1 vial, 15 mL	STOP Stop solution (contains acid). Ready to use.
1 vial, 20 mL	WASH Concentrated wash solution (50x) (Tris, NaN_3 <0.1% w/w). Dilute WASH with distilled or deionized water to a final volume of 1000 mL prior to use.

Material required but not provided:

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipette for 100 µL
- Vortex mixer
- Pipets for 10 µL, 100 µL and 1000 µL
- Laboratory timing device
- Data reduction software
- Distilled or deionized water
- Graduated cylinder for 100 and 1000 mL
- Plastic container for storage of the wash solution

Test procedure

Procedural notes

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination, change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

Sample preparation

Dilute all patient samples **1:100** with DIL before assay.
Therefore combine 10 µL of sample with 990 µL of DIL in a polystyrene tube. Mix well.

1. Pipette **100 µL** of CAL, PC NC and prediluted patient samples in duplicate 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 µL** of diluted WASH.
4. Dispense **100 µL** of CONJ into each well
5. Incubate for **15 minutes** at room temperature
6. Discard the contents of the microwells and wash 3 times with **300 µL** of diluted WASH.
7. Dispense **100 µL** of SUB into each well
8. Incubate for *15 minutes* at room temperature
9. Add **100 µL** of STOP to each well of the modules and incubate for **5 minutes** at room temperature

10. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least **30 minutes**. Read optical densities during this time.

or fully automated on:

- **IASON® PersonalLab**
- **IASON® Quardette**
- **IASON® Gladiator**

Validation

This test is only valid if the optical density at 450 nm for Negative Control NC and Positive Control PC 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.

This assay system is calibrated in relative arbitrary units (IAU = IASON arbitrary units), since no international reference preparation is available for this assay.

Calculation of results

- Draw a standard curve by plotting the absorbance of each standard against its concentration. Read off the values of the test samples. Samples with concentrations higher than that of the highest standard have to be further diluted in DIL or reported as > 100 IAU/mL. For the calculation of the concentrations this dilution factor has to be taken into account.
- Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL (4 parameter logistics) or point-to-point fits are recommended

Expected values

	IAU*/mL
negative	<5
positive	≥5

*IAU= IASON arbitrary units

Each laboratory should establish its individual normal ranges based on results obtained from the local population. Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. The values above should be regarded as guidelines only.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.
Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations.
The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IASON GmbH directly.

Test characteristics

Assay dynamic range

The range of the assay is between 0-100 IAU/mL.

Analytical sensitivity

The analytical sensitivity was and determined to be: 0.5 IAU/mL

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 on the next page.

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 on the next page.

Intra assay cv			Inter assay cv		
Sample	**Mean [IAU/mL]**	**CV [%]**	**Sample**	**Mean [IAU/mL]**	**CV [%]**
A	7.5	6.4	1	7.0	5.0
B	30.2	4.1	2	33.8	4.9
C	59.9	3.1	3	78.3	6.3

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.

Sample	Dilution	Measured Conc. [IAU/mL]	Expected Conc. [IAU/mL]	Recovery (%)
1	1:100	87.3	87.3	100
	1:200	44.1	43.7	101
	1:400	21.5	21.8	99
	1:800	9.7	10.9	89
	1:1600	5.0	5.5	91
2	1:100	79.9	79.9	100
	1:200	39.3	40.0	98
	1:400	19.0	20.0	95
	1:800	8.5	10.0	85
	1:1600	4.3	5.0	86

Study results

Study population	n	n Pos	[%]
Crescendic gromelunophritis	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

		Clinical diagnosis		
		Positive	Negative	
EIASON® pANCA	Positive	54	5	
	Negative	1	145	
		55	150	205

Sensitivity: 98.2 %
Specificity: 96.7 %
Overall agreement: 97.1 %

Limitation of use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

This assay 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 concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

Interfering substances

No interference has been observed with:

- triglycerides up to 3 g/dL
- Bilirubin up to 40 mg/dL
- Haemoglobin up to 1000 mg/dL

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.

Legal aspects

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

REFERENCES / Literature

1. Jennette, J.C. and Falk, R.J. Antineutrophil Cytoplasmic Autoantibodies and Associated Diseases: a Review. Am. J. Kidney Dis. 1990, Vol. XV, No. 6: 517 - 529.
2. Gross, W.L. et al. Antineutrophil Cytoplasmic Autoantibody-Associated Diseases: A Rheumatologist`s Perspective. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 175 - 179.
3. Wieslander, J. How are Antineutrophil Cytoplasmic Autoantibodies Detected ? Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 154 - 158.
4. Lesavre, P. Antineutrophil cytoplasmic antibodies antigen specificity. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 159 - 163.
5. Hagen, E.C. et al. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenic consequences. Blood 1993, Vol.81: 1996 - 2000.
6. Gross,W.L.et al.Immunodiagnostische und immunopathogenetische Bedeutung von Anti-Neutrophilen-Cytoplasma-Antikörpern. Deutsche Medizinische Wochenschrift 1993, Vol. 118: 191 - 199.

Pipetting scheme

Allow all reagents to reach room temperature before use

1. Dilution	Samples 1:100 with DIL
2. Pipetting	CAL 0-5 PC NC diluted samples **100 µL**
3. Incubation	30 min at room temperature (20-28°C)
4. Washing	wash 3 x: aspirate or decant add 300 µL diluted WASH aspirate or decant and dry on an absorbent material
5. Pipetting	CONJ **100µL**
6. Incubation	15 min at room temperature (20-25°C)
7. Washing	wash 3 x : see step 4
8. Pipetting	SUB **100µL**
9. Incubation	15 minutes at room temperature (20-28°C)
10. Pipetting	STOP **100µL**
11. Incubation	5 minutes at room temperature (20-28°C)
12. Reading	450nm (RF 620nm) Optional Overrange Filter: 405nm, Factor: 3 (dependent on photometer), reading within 30 min

Expected values

	IAU*/mL
negative	<5
positive	≥5

* IAU = IASON arbitrary units