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Instruction For Use  
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US Market: For Research Use Only

## ORG 689 ANCAscreen hs (high sensitive)

### NAME AND INTENDED USE

ANCAscreen is an ELISA test system for the qualitative measurement of IgG class autoantibodies against PR3 and MPO in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

### SYMBOLS USED ON LABELS

In vitro diagnostic medical device	MICROPLATE	Microplate
Manufacturer	CONTROL A	Control
Catalogue number	CONTROL B	Control
Sufficient for 96 determinations	CONTROL C	Control
Batch code		
Use by		
Temperature limitation		Sample Buffer P
Consult instructions for use	DILUENT	Enzyme Conjugate
Keep away from sunlight	CONJUGATE	TMB Substrate
Do not reuse	TMB	Wash Buffer
	STOP	Stop solution
Date of manufacture	WASH	
	RTU	Ready to use

### PRINCIPLE OF THE TEST

A mixture of purified antigens PR3 and MPO is coated on to microwells.

Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect 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 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.

### SUMMARY AND EXPLANATION OF THE TEST

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	IF patterns	Target antigen
<i>Systemic Vasculitic Syndromes</i>		
Wegener's Granulomatosis	c-ANCA, rare p-ANCA	PR3, rare MPO
Microscopic Polyangiitis	c-ANCA, p-ANCA	PR3, MPO
Churg-Strauss-Syndrom	p-ANCA	MPO
Polyarteritis nodosa	rare ANCA	rare PR3 and MPO
Unclassified Vasculitis	Rare	no PR3 and MPO
<i>Collagen Diseases and other Rheumatic Disorders</i>		
Rheumatoid arthritis	GS-ANA, p-ANCA, atypical ANCA	unknown, ANA, rare MPO, Lactoferrin
SLE	p-ANCA	rare MPO, Lactoferrin
<i>Other Diseases</i>		
Ulcerative Colitis		Cathepsin-G, Lactoferrin

## CONTENTS OF THE KIT

ORG 689		Sufficient for 96 determinations
<b>MICROPLATE</b>	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: <b>ANC</b>
<b>CONTROL A</b>	1x 1.5 ml	Control A (negative), containing PR3, MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CONTROL B</b>	1x 1.5 ml	Control B (cut-off), containing PR3, MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>CONTROL C</b>	1x 1.5 ml	Control C (positive), containing PR3, MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
<b>DILUENT</b>	15 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
<b>CONJUGATE</b>	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
<b>TMB</b>	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
<b>STOP</b>	15 ml	Stop solution; contains acid. Ready to use.
<b>WASH</b>	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
<b>i</b>	1	Instruction for Use: ELISA Mini-DVD
<b>i</b>	1	Certificate of Analysis

## MATERIALS 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 deionised 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 unopened 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.

## WARNINGS 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.

## PREPARATION OF REAGENTS

### WASH

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

### DILUENT

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

### Preparation of samples

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.

## TEST PROCEDURE

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

- 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.
- Dispense **100 µl** 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.
- Dispense **100 µl** of TMB substrate solution into each well.  
Incubate for **15 minutes** at room temperature
- 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 colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A											
B	B											
C	C											
D	P1											
E	P2											
F	P3											
G												
H												

P1, ... patient sample A-C controls

## 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.

## CALCULATION OF RESULTS

For qualitative results the optical density (OD) of a sample is compared to the optical density of Control B:

Negative: OD sample < OD Control B

Positive: OD sample ≥ OD Control B

For detailed results the optical density of a sample is expressed as Index value:

Index = OD sample / OD Control B

## PERFORMANCE CHARACTERISTICS

### Calibration

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

## Measuring range

not applicable

## 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 Index 1.0

## Interpretation of results

Negative: Index < 1.0

Positive: Index ≥ 1.0

## Linearity

This is a qualitative assay. Requirements for quantitative assays are not applicable.

## Limit of detection

not applicable

## 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 Index	CV %
1 (MPO)	1.3	3.4
2 (PR3)	1.2	5.4
3 (MPO)	1.4	4.1
4 (PR3)	1.1	4.4
5 (MPO)	4.0	3.3
6 (PR3)	4.2	4.5

Inter-Assay		
Sample	Mean Index	CV %
1 (MPO)	1.1	4.6
2 (PR3)	0.9	11.9
3 (MPO)	1.3	9.6
4 (PR3)	1.4	4.5
5 (MPO)	4.6	9.0
6 (PR3)	3.9	7.4

## Interfering substances

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

## Study results

		Comparative Method		
		Pos	Neg	
ORG 689	Pos	39	1	85
	Neg	2	43	
		41	44	
Sensitivity:		95.1 %		
Specificity:		97.7 %		
Overall agreement:		96.5 %		

## LIMITATIONS OF THE PROCEDURE

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.

## REFERENCES

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- 1 Pipet **100 µl** calibrator, control or patient sample  
→ Incubate for **30 minutes** at room temperature  
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 2 Pipet **100 µl** enzyme conjugate  
→ Incubate for **15 minutes** at room temperature  
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 3 Pipet **100 µl** substrate solution  
→ Incubate for **15 minutes** at room temperature
- 4 Add **100 µl** stop solution  
→ Leave untouched for **5 minutes**  
→ Read at **450 nm**