INSTRUCTION MANUAL

REF 4059

February 7, 2008

Anti-PR3

- 96 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to proteinase 3 (PR3) in human serum

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

Anti-PR3 is used for the quantitative or semi-quantitative determination of autoantibodies to proteinase 3 (PR3) in human serum for the differential diagnosis of autoimmune systemic vasculitits (SV).

Pathogenesis of SV is characterized by inflammatory processes of different blood vessel walls and resulting morphological alterations. Both, arteries and veins can be affected simultaneously. Classification of SV by the Chapel Hill Consensus Conference 1992 is based on the kind of blood vessel affected: vasculitides of large vessels (Giant cell arteritis, Takayasu-arteritis), vasculitides of medium vessels (Polyateritis nodosa, Kawasaki-syndrome) and vasculitides of small vessels (Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, Purpura Schönlein-Henoch).

The clinical picture is mainly characterized by general symptoms like exhaustion, fever and weight loss. During the further course of disease symptoms vary dependent on which kind of vessels are affected.
For the serological diagnosis of SV anti-neutrophil cytoplasmic antibodies (ANCA) play an important role. These antibodies are usually determined by indirect immunofluorescence (IIF) of ethanol-fixed human neutrophils. Depending on the pattern of the IIF cytoplasmic ANCA (cANCA) and perinuclear ANCA (pANCA) are distinguished. PR3 has been identified as the responsible autoantigen causing the IIF pattern of cANCA. PR3 autoantibodies are described to be pathognomonic for Wegener's granulomatosis.

The IIF pattern of pANCA is mainly caused by the reactivity of myeloperoxidase (MPO) which is a cationic protein with a molecular weight of 146 kDa found in azurophilic granules. However, other cellular components like lactoferrin, cathepsin G, lysozyme, BPI, and elastase cause pANCA patterns, too and are therefore included in the group of pANCA antigens. MPO autoantibodies occur in a variety of vasculitides such as microscopic polyangiitis, Churg-Strauss syndrome and Polyarteritis nodosa.

Autoantibodies to PR3 are a specific serological marker for Wegener's granulomatosis (WG). Although the etiology and pathogenesis is still obscure, autoantibodies to PR3 are thought to play an active role in the pathogenesis of WG. Anti-PR3 titers are strongly associated with disease activity and inhibit the proteolytic activity of PR3 (2).

(1) Jennete JC, Falk RJ, Andrassy K, Bacon PA, Churg J, Gross WL, Hagen EC, Hoffman GS, Hunder GG, Kallenberg CGM, McCluskey RT, Sinicio RA, Rees AJ, vanEs LA, Waldherr R, Wiik A: Nomenclature of systemic vasculitides: proposal of an international conference. Arthritis Rheum (1994) 37:187-192
(2) Lüdemann J, Utecht B, Gross WL: Antineutrophil cytoplasm antibodies in Wegener's granulomatosis recognize an elastinophil enzyme. J Exp Med (1990) 171: 375-362

PRINCIPLE OF THE TEST

Anti-PR3 is used for the quantitative determination of autoantibodies to proteinase 3 (PR3) in human serum.

The antibodies of calibrators, control and diluted patient samples react with purified human antigens immobilized on the solid phase of microtiter plates. Following an incubation period of 60 min at room temperature, unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve. Evaluating the test by a semi-quantitative method is also possible.

PATIENT SAMPLES

Specimen collection and storage

Blut durch Venenpunktion entnehmen, gerinnen lassen und das Serum durch Zentrifugation isolieren. Lipämische, hämolisierte oder kontaminierte Proben dürfen nicht verwendet werden.
Die Lagerung ist bis zu 3 Tagen bei 2 - 8 °C möglich, darüber hinaus müssen die Proben bei - 20 °C eingefroren werden.
Wiederholtes Einfrieren und Auftauen ist zu vermeiden, ggf. sollte vor dem Einfrieren aliquotiert werden.

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run.

The samples may be kept at 2…8°C for up to three days. Long-term storage requires - 20°C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at – 20°C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v),***
e.g. 10 µl sample + 1.0 ml sample diluent (C), prior to assay.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells coated with with native human PR3 from neutrophil granulocytes	1 vacuum sealed with desiccant
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG- (sheep) coupled with horseradish peroxidase	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 sulfuric acid 0.25 M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml)	1 ml each ready for use capped white
P CONTROL	**Positive control** (diluted serum) conc.: see leaflet enclosed +	1 ml ready for use capped red

Materials required in addition

- Adjustable micropipettes 10 - 100 µl, 100 - 1000 µl
- pipette tips
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- tubes (2 ml) for sample preparation
- distilled or de-ionized water

Size and storage

Anti-PR3 has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-PR3 have to be kept at 2…8°C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable up to 30 days at 2…8 °C.

All other test components are ready for use and stable up to the expiration date printed on each vial.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1+100 (v/v), e.g. 10 µl serum + 1.0 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18…25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 0 – 4 (quantitative) or
   100 µl calibrator 1 (semi-quantitative)
   100 µl positive control (P)
   100 µl diluted patient samples
   into the respective wells.
3. Incubate **60 min** at room temperature (18…25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Incubate **30 min** at room temperature (18…25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min protected from light** at room temperature (18…25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

Anti-PR3 allows both the quantitative (5 calibrators) and semi-quantitative (calibrator 1 for cut-off determination) evaluation of the results.

Quantitative evaluation

We recommend log / lin processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, (lin. scale) versus their respective Anti-PR3 concentrations on the abscissa, x-axis, (log. scale).

Anti-PR3 concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Semi-quantitative evaluation

Results are interpreted by calculating the binding index (BI) using **calibrator 1 (10 U/ml)** as **cut-off control**. The BI is the ratio of the OD-value of a sample to the cut-off OD-value (calibrator 1).

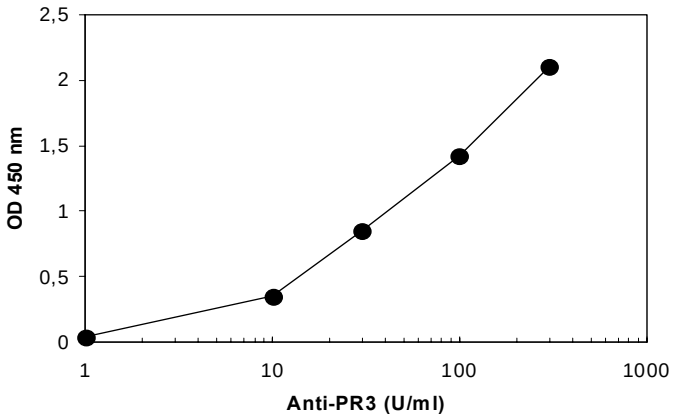
$$\mathbf{BI = OD_{sample} / (OD_{calibrator\ 1})}$$

Both evaluation variants of Anti-PR3 may be achieved also with computer assisted analysis software integrated in the photometers.

Example of Typical Assay Results (quantitative)

Probe	OD (a)	OD (b)	OD (MW)	U/ml
calibrator **0**	0.032	0.048	0.040	**1**
calibrator **1**	0.345	0.355	0.350	**10**
calibrator **2**	0.841	0.863	0.852	**30**
calibrator **3**	1.408	1.435	1.421	**100**
calibrator **4**	2.079	2.124	2.101	**300**
patient **1**	0.788	0.799	0.794	**28**

TYPICAL STANDARD CURVE



Specimens with an OD > calibrator 4 should be diluted with higher volumes of sample diluent and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.7
- the mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Anti-PR3	U/ml	BI
positive	**≥ 10**	**≥ 1.0**
negative	**< 10**	**< 1.0**

It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum Anti-PR3 levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the Anti-PR3. However, anti-PR3 autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA

Calibration

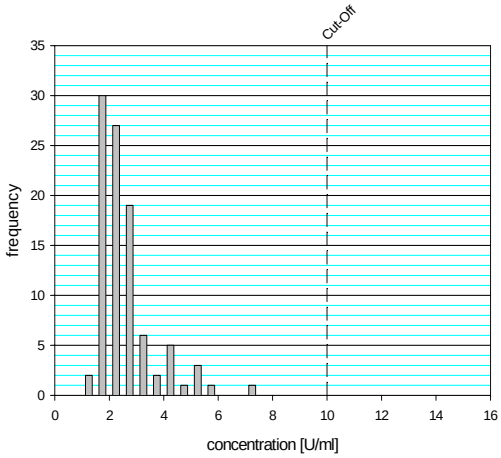
No international reference material for this parameter is available, there for so the assay is calibrated in arbitrary units.

Specificity and sensitivity

The diagnostic sensitivity of the Anti-PR3 is > 95%. No cross reactivity to other autoantigens have been found.

Frequency distribution

97 normal sera (without clinical symptoms) were tested in the Anti-PR3. All sera were negative with concentrations below 8 U/ml.



Precision

Intra-assay			Inter-assay		
sample no.	mean (U/ml)	CV (%)	sample no.	mean (U/ml)	CV (%)
1	195.8	5.96	1	177.5	7.82
2	88.2	4.72	2	101.2	8.81
3	25.6	5.18	3	31.4	8.63
4	16.8	2.47	4	19.2	8.58

INCUBATION SCHEME

Anti-PR3 (4059)

Dilute patients sample	**10 µl serum + 1.0 ml sample diluent (made of C)**

1	Bring all ready for use reagents to room temperature (18…25°C) before use.				
			calibrators	control	sera
2	Pipette	Calibrators (0 - 4) or Calibrator 1	100 µl		
		Positive Control (P)		100 µl	
		prediluted 1 + 100 patient sera			100 µl
3	Incubate		60 minutes at room temperature		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.