

PR3 (c-ANCA) Antibody IgG GENLISA® ELISA

REF : KBD755

Ver 1.2

IVD

Enzyme Immunoassay for Quantitative determination of PR3 (c-ANCA)
Antibody IgG in serum and plasma

IVD	For In-vitro Diagnostic Use	REF	Catalog Number
	Store At	LOT	Batch Code
	Manufactured By		Biological Risk
	Expiry Date		Consult Operating Instructions

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REF KBD755 96 tests**Krishgen Pudgala LLP**Unit Nos#318/319, Shah & Nahar, Off Dr E Moses Road, Worli, Mumbai 400018. India.
Tel: +91-22-49198700 | email: sales@krishgenpudgala.com

Introduction:

Proteinase 3 (PR-3 or PR3) is a small serine protease located in the primary granules and secretory vesicles in the cytoplasm of neutrophils. PR3 is stored in the cytoplasmic granules in resting neutrophils, whilst the expression of the protein on cell surface increases during the activation of neutrophils and during apoptosis. PR3 is also found in neutrophil extracellular traps (NETs) that consist of chromatin released by neutrophils as defense against pathogens.

Autoantibodies against the PR3 are frequently detected in a specific class of autoimmune vasculitides known as Anti-neutrophil cytoplasmic autoantibodies (ANCA) associated vasculitis (AAV). These vasculitides are characterised by the presence of autoantibodies against cytoplasmic components of neutrophils that may have, cytoplasmic staining (c-ANCA) or a particular staining (P-ANCA).

The mechanism of autoimmunity for ANCA antigens is not still being evaluated. Activated neutrophils can undergo apoptosis creating NETs, which are involved in the induction of inflammation. Persistence of NETs during the infection can lead to an autoimmune response. Increased expression of MPO and PR3 in neutrophils and monocytes can influence the pathogenic autoimmune response leading to ANCA-neutrophils activation.

ANCA-associated vasculitides is classified as small vessel vasculitis (SW), which predominantly affect the small vessels, intraparenchymal arteries, arterioles, capillaries and venules. ANCA-associated vasculitides are necrotising vasculitis characterised by a lack of immune deposits in vessel walls causing conditions such as Microscope polyangitis (MPA) and Granulomatosis with polyangitis (GPA, formerly known as Wagner's syndrome).

The frequency of serotype (MPO or PR3) clinical manifestation and geographic distribution of ANCA-associated vasculitides varies, suggesting a role of the environment and the genetic background of patients affected by this category of pathologies.

Intended Use:

The PR3 (c-ANCA) Antibody IgG GENLISA® ELISA is intended for the quantitative determination of PR3 (c-ANCA) Antibody IgG in human serum and plasma from an adult population.

Principle:

The PR3 (c-ANCA) Antibody IgG GENLISA® ELISA is an indirect enzyme linked immunosorbent assay for quantitative determination of PR3 (c-ANCA) Antibody IgG antibody present in the human serum and plasma. The Microtiter wells are pre-coated with specific neutrophil proteinase 3 antigens. Samples and Controls are pipetted into microwells and PR3 (c-ANCA) Antibody IgG present in sample binds to the antigen coated on the wells. Enzyme Conjugate antibody is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution is added to microwells and color develops proportionally to the amount of PR3 (c-ANCA) Antibody IgG present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Coated Microtiter Plate (96 wells) – 1 no
2. Standards (1.2 ml/vial) – 0, 10, 20, 40, 160 AU/ml
3. Positive Control – 1.2 ml
4. Negative Control – 1.2 ml
5. Sample Diluent – 100 ml
6. Enzyme Conjugate – 15 ml
7. (10X) Wash Buffer – 50 ml
8. TMB Substrate – 15 ml
9. Stop Solution – 15 ml
10. Instruction Manual – 1 no.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul.
3. Deionized (DI) water.
4. Wash bottle or automated microplate washer.
5. Graph paper or software for data analysis.
6. Timer.
7. Absorbent Paper.

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Sample Preparation and Storage:**

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

1. Extract as soon as possible after specimen collection as per relevant procedure. The samples should be tested as soon as possible after the extraction. Alternately the extracted samples can be kept in -20°C. Avoid repeated freeze-thaw cycles.
2. Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.
3. Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.
Note: Grossly hemolyzed samples are not suitable for use in this assay.

Preparation of Samples:

All serum or plasma samples must be prediluted 1:100 with sample diluent; for example, 10 ul of sample should be diluted with 990 ul of sample diluent.

Reagent Preparation:

1. Allow all components to reach 2-8°C prior to use in the assay.
2. (1X) Wash Buffer Dilution: To make (1X) Wash Buffer, add 50 ml of (10X) Wash Buffer to 450 ml of DI water. This is the working solution.

Test Procedure:

1. Add **100 ul Diluted Sample, Standards, Positive Controls and Negative Control** into appropriate wells.
2. Incubate at Room Temperature for **60 minutes**.
3. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.

4. Add **100 ul Enzyme Conjugate** into each well, except blank. Mix well.
5. Incubate at Room Temperature for **30 minutes**.
6. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7. Add **100 ul of TMB Substrate** to all wells.
8. Incubate at Room Temperature for 15 minutes.
9. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 5 minutes of stopping the reaction.

Calculation of Results:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown PR3 (c-ANCA) Antibody IgG concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the PR3 (c-ANCA) Antibody IgG Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a 4-PL (2nd order), or cubic spline is best recommended for automated results.

Validity of the Test:

The test is valid when the controls fall in below given range:

Negative Control: < 16 AU/ml

Positive Control: ≥ 20 AU/ml

Standards: 16 AU/ml – 20 AU/ml

Measuring Range:

The assay measuring range (AMR) is 3.2-160 AU/ml

Any value that reads below 3.2 AU/ml should be reported as "< 3.2 AU/ml". Any value that reads above 160 AU/ml should be reported as "> 160 AU/ml".

Metrology And Traceability:

The calibrators of this kit are traceable to the Centres for Disease Control and Prevention (CDC). Human Reference Serum #16 for Anti-Proteinase 3 Ab.

Expected Values:

The following ranges were determined using the PR3 (c-ANCA) Antibody IgG assay and are provided for information only. The 90% reference interval for apparently healthy adults were calculated by a non-parametric method following guidance from CLSI C28-A3 "Defining Establishing and Verifying Reference Intervals in the Clinical Laboratory").

No. of subjects	73
Mean (AU/ml)	8.71
SD (AU/ml)	3.21
Median (AU/ml)	8.12
Reference Interval (AU/ml)	4.19-14.40

The above ranges should be considered as guidelines only. It is recommended that each laboratory establish its own expected range based upon its own patient population.

Interpretation of Results:

Concentration	Interpretation
< 16 AU/ml	The sample should be considered negative
16-20 AU/ml	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
≥ 20 AU/ml	The sample should be considered Positive

The above index and interpretation should be considered as guidelines only. Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken on an individual patient basis.

It is recommended that each laboratory establishes its own normal and pathological ranges of anti-PR3.

Performance Characteristics:

Representative performance data are shown. Results obtained at individual laboratories may vary.

Detection Capability:

The limit of blank (LoB) limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.6 AU/ml
Limit of Detection (LoD)	1.6 AU/ml
Limit of Quantitation (LoQ)	3.2 AU/ml

Trueness:

Trueness has been demonstrated through a recovery test of the PR3 (c-ANCA) Antibody IgG assay with the human Anti-Proteinase 3 reference serum #16 from the centres for Disease Control and Prevention (CDC).

Precision:

Precision of the PR3 (c-ANCA) Antibody IgG assay was determined by performing a complex precision study.

Repeatability:

A total of 6 samples were assayed in 5 replicates once a day for 5 days by 3 operators. Data from one representative lot is shown below,

Sample	n	Mean Conc. (AU/ml)	Within run (Repeatability)	
			SD	CV%
1	75	16.51	0.96	5.8%
2	75	23.94	0.93	3.9%
3	75	40.03	1.23	3.1%
4	75	59.68	3.40	5.7%
5	75	86.96	2.80	3.2%
6	75	136.70	6.24	4.6%

Reproducibility:

A total of 6 samples were assayed in 5 replicates once a day for 5 days by 3 operators. Results for the combined data from two lots are shown below.

Sample	n	Mean Conc. (AU/ml)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	16.57	1.49	9.0%
2	150	23.95	1.53	6.4%
3	150	40.59	2.67	6.6%
4	150	60.11	4.15	6.9%
5	150	86.69	6.12	7.1%
6	150	137.19	9.63	7.0%

Linearity:

Linearity was established based on CLSI EP-06. "Evaluation of the Linearity of Quantitative Measurement Procedures". For Anti- PR3 concentration by PR3 (c-ANCA) Antibody IgG assay, the measurement procedure shows linearity for the interval from 1.73 to 162.62 AU/ml within the allowable derivation of linearity (ADL) of $\pm 15\%$.

Diagnostic Sensitivity and Specificity:

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristics Curves". Using 73 negative and 50 positive samples run on a single run on a single reagent lot.

		Positive	Negative	Total
True	Positive	50	0	50
State	Negative	2	71	73
Total		52	71	123

Sensitivity: 100%

Specificity: 97.3%

Interference:

The following substances do not interfere with a bias of $> \pm 15\%$ in the PR3 (c-ANCA) Antibody IgG assay when the concentrations are below the stated presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin Conjugated	15 mg/dl
Bilirubin Unconjugated	15 mg/dl
Haemoglobin	200 mg/dl
Total Protein	10 g/dl
Triglycerides	500 mg/dl

Serum-plasma study:

The Anti-PR3 (c-ANCA) assay matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST) lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples (16 native, 4 spiked or diluted) to cover the range of 2.80 to 74.40 AU/ml were evaluated. Linear regression analysis was performed on the comparative data.

Sample Type	Slope (95% CI)	Intercept (AU/ml) (95% CI)	Correlation Coefficient (r)
SST	1.0 (0.97 to 1.08)	0.14 (-1.17 to 1.45)	0.99
Lithium Heparin	1.1 (1.06 to 1.21)	-0.46 (-2.09 to 1.17)	0.99
Sodium Heparin	1.1 (1.05 to 1.14)	-0.91 (-1.88 to 0.06)	1.00
EDTA	1.1 (1.0 to 1.12)	0.00 (-1.79 to 1.78)	0.99

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for 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 in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

**LIMITED WARRANTY**

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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Unit No.1/2, Om Sainath Commercial Complex,
Off Mankoli-Anjur Phata Road. Village Dapode, Bhiwandi 421302.

Regulatory Status:

CE Marked	Europe
FDA registered	USA
CDSCO registered	India

SCHEMATIC ASSAY PROCEDURE

1	Add 100 ul Diluted Sample, Standards, Positive Controls and Negative Controls into appropriate wells.
2	Incubate at Room Temperature for 60 minutes.
3	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
4	Add 100 ul Enzyme Conjugate into each well, except blank. Mix well.
5	Incubate at Room Temperature for 30 minutes.
6	Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
7	Add 100 ul of TMB Substrate to all wells.
8	Incubate on shaker at Room Temperature for 15 minutes .
9	Add 100 ul of Stop Solution . Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

SYMBOLS KEY

	Microtiter Plate (96 wells)
	Standards
	Positive Control
	Negative Control
	Enzyme Conjugate
	Sample Diluent
	(10X) Wash Buffer
	TMB Substrate
	Stop Solution
	Consult Instructions for Use
	Catalog Number
	Expiration Date
	Storage Temperature