

# QUANTA Lite™ PR-3 IgG ELISA

708705

For *In Vitro* Diagnostic Use

CLIA Complexity: High

## Intended Use

QUANTA Lite™ PR-3 is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgG autoantibodies to serine protease 3 (PR-3) in human serum. This test is to be used in conjunction with other clinical findings to aid in assessment of certain autoimmune vasculitides such as Wegener's granulomatosis.

## Summary and Explanation of the test

Anti-neutrophil cytoplasmic antibody (ANCA) testing has revolutionized the diagnosis and treatment of the various autoimmune mediated vasculitides.<sup>1-4</sup> The pANCA and cANCA autoantibodies have proven to be useful clinically and interesting scientifically for the detection of diseases such as Wegener's granulomatosis (WG) and crescentic glomerulonephritis.

There are at least six identified ANCA antigens and many are still unidentified.<sup>1,5</sup> Most of these antigens appear to be enzymes residing in the neutrophil primary granules. These enzymes include myeloperoxidase (MPO), serine protease 3 (PR-3), elastase, lactoferrin, cathepsin G and cationic protein 57 (CAP-57). Earlier review articles have stated that 80-90% of cANCA samples have reactivity to PR-3.<sup>1,3</sup> The actual percentage depends on the patient population studied and the quality of the ELISA procedure used. Many ELISA methods were found to have contaminants in the "purified" antigen used to coat the solid phase resulting in false positive results. Our own studies with very clean and specific ELISA methods are more in the 50-60% range. Most experts in the field of autoimmune vasculitis are still recommending that the IFA be used for initial screening. Follow-up testing with specific MPO and PR-3 ELISA assays of all IFA positive samples can provide additional information.

In a recent study including 277 patients with WG and 1657 control patients, the specificity of anti-PR-3 antibodies for WG was determined to be 98%. PR-3 was found in 93% of patients with active generalized disease, in 60% of patients with active regional disease and in 40% of patients in remission. It has been demonstrated that autoantibody changes parallel disease activity and help to distinguish relapses of WG from other intercurrent illness (such as infections) which are always a threat to those on immunosuppressive therapy.<sup>6</sup>

Anti-MPO antibodies are highly specific for idiopathic and vasculitis associated crescentic glomerulonephritis and also for classic polyarteritis nodosa, Churg-Strauss syndrome and the polyangitis overlap syndrome without renal involvement.<sup>7-10</sup> With respect to sensitivity, either MPO or PR-3 antibodies were found in 77 to 100% of patients with idiopathic and vasculitis associated crescentic glomerulonephritis. In WG, anti-MPO antibodies were detected only occasionally and generally in patients negative for PR-3 antibodies.<sup>7</sup> Levels of MPO antibodies are significantly higher during active phases of disease compared to phases of remission.<sup>7</sup> Therefore, these autoantibodies, like PR-3 antibodies appear to be markers for disease activity.

MPO and PR-3 ELISA methods cannot replace the standard IFA using human neutrophils for detecting ANCA, as there are too many other specificities that are important. This is especially true in the case of atypical or inflammatory bowel disease (IBD) ANCA found in patients with ulcerative colitis and sclerosing cholangitis.<sup>4</sup> The MPO and PR-3 specific ELISA methods can provide an important confirmatory result for two of the more important of the identified antigens. ELISA is also useful for interpreting "difficult" samples by IFA such as those which exhibit several antibodies simultaneously or those with high background fluorescence. The ELISA technique employed in this test is sensitive, specific and objective. It can be conveniently used to test both large and small numbers of samples.

## Principles of the Procedure

Purified human PR-3 antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any PR-3 antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies, which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

## Reagents

1. Polystyrene microwell ELISA plate coated with a purified PR-3 antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human IgG antibodies to PR-3, prediluted, 1.2mL
3. PR-3 IgG ELISA Low Positive, 1 vial of buffer containing preservative and human serum IgG antibodies to PR-3, prediluted, 1.2mL
4. PR-3 IgG ELISA High Positive, 1 vial of buffer containing preservative and human serum IgG antibodies to PR-3, prediluted, 1.2mL

5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.
7. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

## Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.
2. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the PR-3 IgG ELISA Low Positive, PR-3 IgG ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.<sup>11</sup>
3. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

## Precautions

1. This product is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing and insufficient liquid removal from the ELISA well strips will cause poor precision and/or high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

## Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week at 2-8°C.

## Specimen Collection

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS Document H18-A2 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hrs, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

## Procedure

### Materials provided

- 1 PR-3 IgG ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ELISA Negative Control
- 1 1.2mL prediluted PR-3 IgG ELISA Low Positive
- 1 1.2mL prediluted PR-3 IgG ELISA High Positive
- 1 50mL HRP Sample Diluent
- 1 25mL HRP Wash Concentrate, 40x concentrate
- 1 10mL HRP IgG Conjugate, (goat), anti-human IgG
- 1 10mL TMB Chromogen
- 1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

### Additional Materials Required But Not Provided

- Micropipets to deliver 5, 100, 200-300 and 500µL
- Disposable micropipet tips
- Test tubes for patient sample dilutions, 4mL volume
- Distilled or deionized water
- 1L container for diluted HRP Wash Concentrate
- Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

## Method

### Before you start

- 1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
- 2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
- 3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the PR-3 IgG ELISA Low Positive, PR-3 IgG ELISA High Positive and ELISA Negative Control.
- 4. Determination of the presence or absence of PR-3 using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

### Assay procedure

- 1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.**
- 2. Add 100µL of the **prediluted** PR-3 IgG ELISA Low Positive, the PR-3 IgG ELISA High Positive, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
- 3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the **diluted** HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
- 4. Add 100µL of the HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 2.
- 5. Wash step: Repeat step 3.
- 6. Add 100µL of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
- 7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
- 8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

## Quality Control

1. The PR-3 IgG ELISA Low Positive, the PR-3 IgG ELISA High Positive and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the PR-3 IgG ELISA Low Positive, the PR-3 IgG ELISA High Positive and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at  $\leq -20^{\circ}\text{C}$ .
4. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated.
  - a. The absorbance of the prediluted PR-3 IgG ELISA High Positive must be greater than the absorbance of the prediluted PR-3 IgG ELISA Low Positive, which must be greater than the absorbance of the prediluted ELISA Negative Control.
  - b. The prediluted PR-3 IgG ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
  - c. The PR-3 IgG ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
  - d. The ELISA Negative Control and PR-3 IgG ELISA High Positive are intended to monitor for substantial reagent failure. The PR-3 IgG ELISA High Positive will not ensure precision at the assay cutoff.
  - e. The user should refer to NCCLS Document C24-A for additional guidance on appropriate QC practices.

## Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the PR-3 IgG ELISA Low Positive. The result is multiplied by the number of units assigned to the PR-3 IgG ELISA Low Positive found on the label.

$$\text{Sample Value (units)} = \frac{\text{Sample OD}}{\text{PR-3 IgG ELISA Low Positive OD}} \times \text{PR-3 IgG ELISA Low Positive (units)}$$

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient's antibody titer.

## Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

The sample can then be classified as negative, weak positive, moderate positive or strong positive according to the table below.

Negative	Units $\leq 20$
Weak Positive	21 – 30
Moderate Positive to Strong Positive	$>30$

1. A positive result indicates the presence of PR-3 antibodies and suggests the possibility of certain autoimmune vasculitides such as Wegener's granulomatosis.
2. A negative result indicates no PR-3 antibody or levels below the negative cut-off of the assay.
3. It is suggested that the results reported by the laboratory should include the statement: "The following results were obtained with the INOVA QUANTA Lite™ PR-3 IgG ELISA. PR-3 values obtained with different manufacturers' assay methods may not be used interchangeably. The magnitude of the reported IgG levels cannot be correlated to an endpoint titer."

## Limitations of the Procedure

1. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
2. Results of this assay should be used in conjunction with clinical findings and other serological tests including ANCA by indirect immunofluorescence.
3. Results of this assay are not diagnostic proof of the presence or absence of disease. Immunosuppressive therapy should not be initiated based solely on positive results.
4. The assay performance characteristics have not been established for matrices other than serum.

## Expected Values

The ability of the QUANTA Lite™ PR-3 IgG ELISA to detect PR-3 antibodies was evaluated by comparison to a commercially available ELISA test. Results of the ELISA test were determined according to the manufacturer's direction insert.

## Normal Range

One hundred random normal samples were run. All were less than the 20 unit cutoff for PR-3. Mean PR-3 result was 1.4 units with the highest reactive sample having a value of 4 units.

## PR-3 Autoantibodies in Various Disease Groups

Group	Number	Number positive	(%)
Normals	100	0	(0)
Non-ANCA Kidney Disease	66	3	(4.5)
Wegener's (cANCA Pos.)	43	43	(100)
Crescentic Glomerulonephritis (pANCA Pos.)	45	0	(0)

All 100 normal samples were negative and all 43 cANCA positive Wegener's patients were positive. None of the 45 pANCA positive samples were detected as PR-3 positive. Three patients out of 66 total forms the "non-ANCA kidney disease" group, which was detected as positive. This latter group of 66 samples included patients with anti-glomerulo basement membrane (GBM) disease, Lupus glomerulonephritis, thrombotic microangiopathy, IgA nephropathy and immune complex disease.

## Relative Sensitivity and Specificity

Samples from the above mentioned Wegener's and crescentic glomerulonephritis groups as well as the normals and the 66 sample non-ANCA disease group were tested by both the QUANTA Lite™ PR-3 IgG ELISA kit and by another reference ELISA using polyvalent conjugate. The results appear below.

		INOVA			
		+	-		
Reference	+	21	35*	Relative Sensitivity	37.5%
	-	1	149	Relative Specificity	99.3%
				Relative Efficiency	82.5%

\* The majority of these samples were from the non-ANCA kidney disease group.

## Precision and Reproducibility

The precision and reproducibility of the assay was measured by running six replicates each of negative, weak positive and strong positive samples in six separate assays. The mean of the strong positive was 100.1, the weak positive was 25 and the negative was 18.75. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Weak Positive	
	SD	CV	SD	CV	SD	CV
Overall	0.81	4.3%	8.90	8.9%	0.80	3.2%
Within Run	0.60	3.2%	2.01	2.0%	0.83	3.3%
Between Run	0.79	4.2%	9.40	9.4%	0.57	2.3%

## References

1. Goeken JA: Antineutrophil cytoplasmic antibody - a useful serological marker for vasculitis. *J Clin Immunol* **11**:161-174, 1991.
2. Specks U, et al.: Anticytoplasmic autoantibodies in the diagnosis and follow-up of Wegener's granulomatosis. *May Clin Proc* **64**:28-36, 1989.
3. van der Woude FJ, et al.: Autoantibodies against neutrophils and monocytes: tools for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* **423**:429, 1985.
4. Cohen-Tervaert JW, et al.: Association between active Wegener's granulomatosis and anticytoplasmic antibodies. *Arch Intern Med* **149**:2461-2465, 1989.
5. Cambridge, et al.: Antineutrophil antibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* **33**:668-679, 1992.
6. Nolle B, et al.: Anticytoplasmic autoantibodies: their immunodiagnostic value in Wegener's granulomatosis. *Ann Int Med* **111**:28-40, 1988.
7. Cohen-Tervaert JW, et al.: Association of autoantibodies to myeloperoxidase with different forms of vasculitis. *Arthritis and Rheumatism* **33**(8):1264-1272, 1990.
8. Cohen-Tervaert JW, et al.: Autoantibodies against myeloid lysosomal enzymes in crescentic glomerulonephritis. *Kidney Int* **37**:799-806, 1990.
9. Falk RJ and Jenette JC: Anti neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* **318**:1651-1657, 1988.
10. Cohen-Tervaert JW, et al.: Detection of autoantibodies against myeloid lysosomal enzymes: a useful adjunct to classification of patients with biopsy proven necrotizing arteritis. *Am J Med* **91**:59-66, 1991.
11. *Biosafety in Microbiological and Biomedical Laboratories*. Centers for Disease Control/National Institute of Health, 2007, Fifth Edition.

Manufactured By:

INOVA Diagnostics, Inc.  
9900 Old Grove Road  
San Diego, CA 92131  
United States of America

Authorized Representative in the EU:

Medical Technology Promedt Consulting GmbH  
Altenhofstrasse 80  
D-66386 St. Ingbert, Germany  
Tel.: +49-6894-581020  
Fax.: +49-6894-581021  
[www.mt-procons.com](http://www.mt-procons.com)

Technical Service  
628705USA

888-545-9495  
September 2009  
Revision 6

