ELISA PRODUCT INFORMATION & MANUAL

Proteinase 3 (PR3) Autoantibody

NBP2-60594

Enzyme-linked Immunosorbent Assay for quantitative detection of Human Proteinase 3 (PR3) Autoantibody.
For research use only.
Not for diagnostic or therapeutic procedures.

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Assay Summary

Step 1. Add 50 µl of Standard or Sample per well.
Incubate 2 hours.

Step 2. Wash, then add 50 µl of HRP Conjugate per well.
Incubate 1 hour.

Step 3. Wash, then add 50 µl of Chromogen Substrate per well.
Incubate 30 minutes.

Step 4. Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
1								
2								
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Human Proteinase 3 Autoantibody ELISA Kit (Anti-Proteinase 3 IgG)

Catalog No. NBP2-60594

Sample insert for reference use only

Introduction

Proteinase 3 (PR3, PRTN3), a neutral serine proteinase, is also known as myeloblastin, Wegener autoantigen, azurophil granule protein 7, and neutrophil protease p29 (1). PR3 is produced and packaged into azurophil granules during neutrophil differentiation. The mature PR3 consists of 228 amino acids and has a molecular mass of approximately 29 kDa (2). PR3 degrades connective-tissue proteins, in particular elastin, fibronectin, type IV collagen, and laminin (3). It has potent antimicrobial activity and is involved in a variety of immune defense reactions that contribute to the destruction of ingested microorganisms. Autoantibodies to PR3 are involved in the pathogenesis of autoimmune-mediated vasculitis in granulomatosis with polyangiitis (4).

Principle of the Assay

The Human Proteinase 3 Autoantibody ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative determination of autoimmune response (IgG) to a target antigen (Proteinase 3). The kit detects autoantibodies in **plasma and serum samples**. This assay employs a quantitative **sandwich enzyme immunoassay technique that measures autoantibodies (anti-Proteinase 3 IgG) in less than 4 hours**. A proteinase 3 antigen has been pre-coated onto a 96-well microplate with removable strips. Autoantibody specific for proteinase 3 in standards and samples is sandwiched by the immobilized antigen and an antibody-HRP conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This product is for **Research Use Only** and is not intended for use in diagnostic procedures.
- Prepare all reagents (working diluent buffer, wash buffer, standard, HRP conjugate) as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.

- Spin down the HRP conjugate vial before opening and using contents.
- The Stop Solution is an acidic solution.
- The kit should not be used beyond the expiration date.

Reagents

- **Human Proteinase 3 Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with proteinase 3.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Proteinase 3 Standard:** Plasma standard (15 AU, lyophilized).
- **HRP Conjugate (50x):** A 50-fold concentrated HRP-antibody conjugate (120 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 1 bottle).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store HRP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation, and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:40 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:40 into EIA Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **EIA Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the EIA Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the Standard 15 AU with 0.5 ml of EIA Diluent to generate a 30 AU/ml standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate points by serially diluting the standard stock solution (30 AU/ml) 1:2 using equal volume of EIA Diluent to produce 15, 7.5, 3.75, and 1.875 AU/ml solutions. EIA Diluent serves as the zero standard (0 AU/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Proteinase 3] (AU/ml)
P1	1 part Standard (30 AU/ml)	30.00
P2	1 part P1 + 1 part EIA Diluent	15.00
P3	1 part P2 + 1 part EIA Diluent	7.500
P4	1 part P3 + 1 part EIA Diluent	3.750
P5	1 part P4 + 1 part EIA Diluent	1.875
P6	EIA Diluent	0.000

- **HRP Conjugate (50x):** Spin down the HRP Conjugate briefly and dilute the desired amount of the conjugate 1:50 with EIA Diluent. Any remaining solution should be frozen at -20°C.

- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
- Add 50 µl of HRP Conjugate to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for 30 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve.

- Although normal samples have been diluted 1:40, do not multiply the value by the dilution factor. Samples with elevated level of autoantibodies can be diluted further; for example 1:80. Account for this further dilution factor when calculating the value of the sample.

Example	Dilution Factor	Multiplication Factor For Calculating Values
Serum with normal level of anti-Proteinase 3 IgG	40x	1
Serum with elevated level of anti-Proteinase 3 IgG	80x	2
Serum with elevated level of anti-Proteinase 3 IgG	160x	4

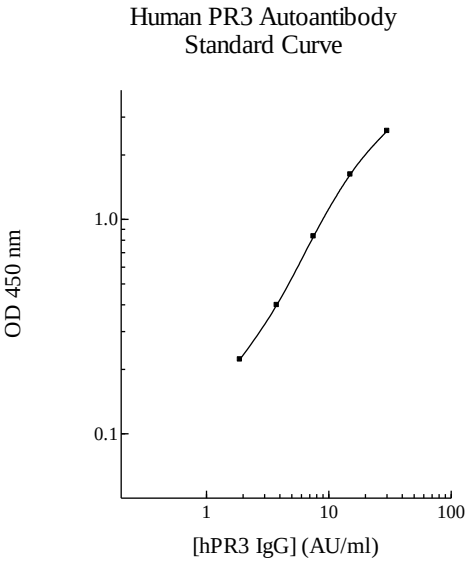
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	AU/ml	Average OD
P1	30.00	2.586
P2	15.00	1.621
P3	7.500	0.832
P4	3.750	0.398
P5	1.875	0.222
P6	0.000	0.069
Normal Level Sample (40x): Serum with normal level of anti-Proteinase 3 IgG		0.463
Elevated Level Sample (80x): Serum with elevated level of anti-Proteinase 3 IgG		0.921

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Reference Value

- Human plasma and serum samples from healthy adults were tested (n=20). Moreover, patient serum samples containing high levels of anti-proteinase 3 IgG were tested (n=12). The following ranges have been established:

Sample	Anti-Proteinase 3 IgG (AU/mL)
Normal Level	< 10.0
Elevated Level	≥ 10.0

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

Performance Characteristics

- The minimum detectable dose of autoantibodies as calculated by 2SD from the mean of a zero standard was established to be 1.0 AU/ml.
- Intra-assay precision was determined by testing replicates of three plasma samples in one assay.
- Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.8%	4.1%	3.9%	7.8%	8.2%	8.9%
Average CV (%)	3.9 %			8.3%		

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of expired components	• Check the expiration date listed before use. • Do not interchange components from different lots.
	Improper wash step	• Check that the correct wash buffer is being used. • Check that all wells are dry after aspiration. • Check that the microplate washer is dispensing properly. • If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	• Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.
	Improperly sealed microplate	• Check the microplate pouch for proper sealing. • Check that the microplate pouch has no punctures. • Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	• Each step of the procedure should be performed uninterrupted.
	Omission of step	• Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	• Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	• Check pipette calibration. • Check pipette for proper performance.
	Wash step was skipped	• Consult the provided procedure for all wash steps.
	Improper wash buffer	• Check that the correct wash buffer is being used.
	Improper reagent preparation	• Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	• Consult the provided procedure for correct incubation time.
Deficient Standard Curve Fit	Non-optimal sample dilution	• Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. • Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. • User should determine the optimal dilution factor for samples.

	Contamination of reagents	• A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	• Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	• Pipette properly in a controlled and careful manner. • Check pipette calibration. • Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	• Thoroughly agitate the lyophilized components after reconstitution. • Thoroughly mix dilutions.

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