ab284018 – Human PR3 Autoantibody ELISA Kit

For the quantitative measurement of PR3 in human plasma and serum
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab284018

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the SP Conjugate, the 50X Biotinylated Human PR3 Autoantibody Antibody, and the Human PR3 Autoantibody Microplate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µl	-20°C
10X Diluent M Concentrate	30 ml	+4°C
20X Wash Buffer Concentrate	2 x 30 µl	+4°C
50X Biotinylated Human PR3 Autoantibody Antibody	1 vial	-20°C
Chromogen Substrate	7 mL	+4°C
Human PR3 Autoantibody Microplate	96 wells	-20°C
PR3 Autoantibody Standard	1 vial	+4°C
Sealing Tapes	3	+4°C
Stop Solution	11 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
Prepare only as much reagent as is needed on the day of the experiment.
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

Diluent M Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Diluent M Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2-8°C.

Human Proteinase 3 Autoantibody Standard: Reconstitute the Human Proteinase 3 Autoantibody Standard (18 AU) with 0.6 ml of Diluent M to generate a 30 AU/ml standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (30 AU/ml) 2-fold with equal volume of Diluent M to produce 15, 7.5, 3.75, and 1.875 AU/ml solutions. Diluent M serves as the zero standard (0 AU/ml). Aliquot remaining stock solution to limit repeated freeze thaw cycles. This solution should be stored atm-20°C and used within 30 days.

Biotinylated Human PR3 Autoantibody Antibody (50x): Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with Diluent M to produce a 1x solution. The undiluted antibody should be stored 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 20-fold with reagent grade water to produce a 1x solution.

SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Prepare serially diluted standards immediately prior to use.
Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).
Reconstitution of the Human PR3 Autoantibody Standard vial to prepare a 30 AU/mL Stock Standard.

- First consult the Human PR3 Autoantibody Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of 1X Diluent M to add when resuspending the Human PR3 Autoantibody Standard vial to produce a 30 AU/mL Human PR3 Autoantibody Stock Standard by using the following equation:
    - C_S = Starting mass of Human PR3 Autoantibody Standard (see vial label) (AU/ml)
    - C_F = The 30 AU/ml Human PR3 Autoantibody Stock Standard final required concentration
    - V_D = Required volume of 1X Diluent M for reconstitution (µL)
    - Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example: **Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided**.

CS = 21 AU of Human PR3 Autoantibody Standard in vial

CF = 30 AU/mL Human PR3 Autoantibody Standard #1 final concentration

VD = Required volume of 1X Diluent N for reconstitution (21 AU / 30 AU/mL) x 1,000 = 700 µL

- Reconstitute the Human PR3 Autoantibody Standard vial by adding the appropriate calculated amount VD of 1X Diluent M to the vial to generate the 30 AU/mL Human PR3 Autoantibody Standard #1. Mix gently and thoroughly.
- Allow the reconstituted 10 AU/mL Human PR3 Autoantibody Standard #1 to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label four tubes #2 – 6.
- Add 120 µL of 1X Diluent M to tube #2 – 6.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently.

- To prepare Standard #3, add 120 µL of the Standard #2 into tube #3 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. 1X Diluent M serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	PR3 (AU/mL)
1	240 µL	-	30
2	120 µL Standard #1	120 µL	15
3	120 µL Standard #2	120 µL	7.5
4	120 µL Standard #3	120 µL	3.75
5	120 µL Standard #4	120 µL	1.875
6	-	120 µ	0.0

Sample Preparation

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 and collect plasma.
A 40-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs.
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.
A 40-fold sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs.
The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

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).

1. 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 at -20°C.
2. Add 50 µl of Human Proteinase 3 Autoantibody Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
3. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
4. Add 50 µl of Biotinylated Human PR3 Autoantibody Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
5. Wash the microplate as described above.
6. Add 50 µl of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
7. Wash the microplate as described above.
8. Add 50 µl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops.
9. Add 50 µl of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
10. 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.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the standard curve.
4. Although normal samples have been diluted 40-fold, do not multiply the value by the dilution factor. Samples with elevated levels of autoantibodies can be further diluted; for example: 80x. Account for this further dilution factor when calculating the value of the sample.

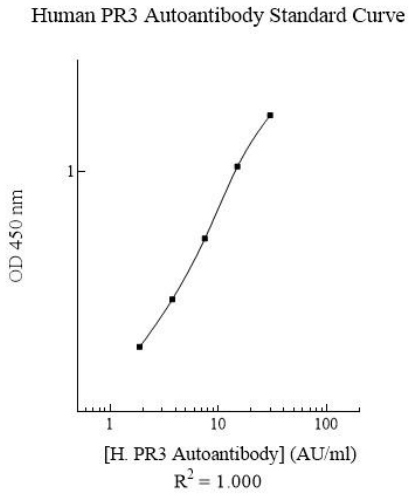
TYPICAL DATA

Typical data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Point	AU/ml	Average OD
P1	30	2.220
P2	15	1.424
P3	7.5	0.821
P4	3.75	0.446
P5	1.875	0.255
P6	0.0	0.042
Normal Level Sample (40x): Serum with normal level of anti-PR3 IgG		0.217
Elevated Level Sample (80x): Serum with elevated level of anti-PR3 IgG		0.765

STANDARD CURVE

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



PERFORMANCE CHARACTERISTICS

1. The minimum detectable dose of anti-PR3 IgG as calculated by 2SD from the mean of a zero standard was established to be 0.44 AU/ml.
2. Intra-assay precision was determined by testing three serum samples ten times in one assay.
3. Inter-assay precision was determined by testing three serum samples in ten assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	6.4%	9.6%

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide
For all technical or commercial enquiries please go to:
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