ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Proteinase 3 (PR3) ELISA Kit

(Catalog Number: 31300)

For the quantitative determination of human proteinase 3 (PR3) in serum, plasma, cell culture media or other biological samples

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INTRODUCTION

Proteinase 3 (PR3), also known as myeloblastin, Wegener autoantigen, PRTN3 and NP-4, is one of the hematopoietic serine proteases localized in the primary granules of polymorphonuclear neutrophils (PMNs)[1,2]. The primary function of PR3 is recognized as to participate in direct intracellular killing of phagocytosed pathogens in phagolysosomes and degradation of extracellular matrix components at inflammatory sites[3]. PR3 has also been proven to be able to process some pro-inflammatory cytokines, such as IL1β, IL-18 and TNF-α, activate mitogen activated protein kinase (MAPK) signaling through proteinase activated receptor-1 (PAR1), and induce endothelial cell apoptosis through NF-κB signaling pathways[4,5]. PR3 is identified as the target autoantigen of anti-neutrophil cytoplasmic autoantibodies (ANCA) in Wegener granulomatosis[6]. Increased PR3 levels have been reported in patients with acute myocardial infarction[7], and in subjects with type 1 diabetes[8].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter strips are pre-coated with a polyclonal antibody specific for human PR3. Standards and samples are pipetted into the wells and any human PR3 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP) labelled polyclonal antibody specific for human PR3 is added to the wells. After wash step to remove any unbound reagents, an HRP substrate solution is added and color develops in proportion to the amount of human PR3 bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increase in absorbance is directly proportional to the amount of captured human PR3, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human PR3 ELISA kit is designed for quantification of human PR3 in serum, plasma, cell culture media and other biological samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a polyclonal antibody against human PR3, sealed
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, a polyclonal antibody against human PR3 conjugated to horseradish peroxidase, 0.12 mL
5. Human PR3 standard, 10 ng of recombinant human PR3, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

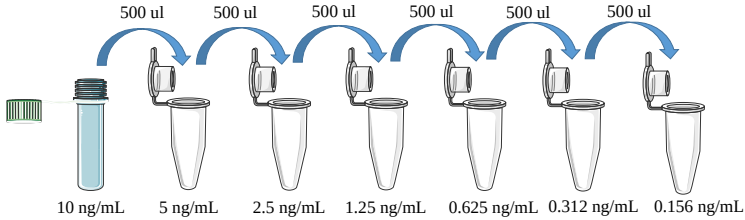
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 28°C immediately after the necessary volume is pipetted.

PREPARATION OF STANDARDS AND SAMPLES

Human PR3 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 10 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC for up to one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **100-fold** dilution in 1×Assay buffer. A suggested dilution step is to add 10 µL of sample to 990 µL of 1×Assay buffer. If a sample has a PR3 level greater than the highest standard, the sample should be diluted further, and the assay should be repeated. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human PR3 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

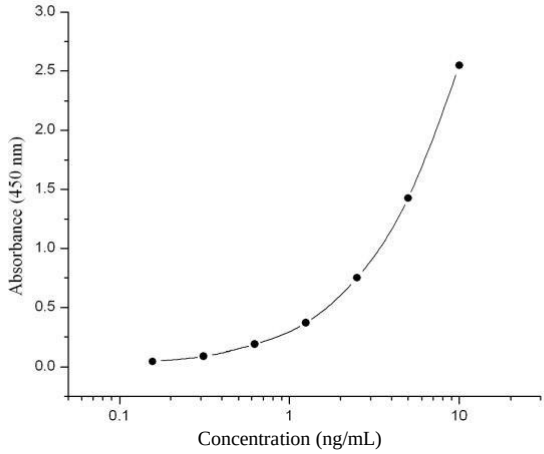
3. Determine human PR3 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

PR3 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.056	0
0.156	0.102	0.046
0.312	0.145	0.089
0.625	0.245	0.189
1.25	0.426	0.37
2.5	0.808	0.752
5	1.482	1.426
10	2.603	2.547

Human PR3 standard curve (4-parameter)



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SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation