



EAGLE
BIOSCIENCES

Human Proteinase 3 ELISA Kit

Catalog Number:

PRT31-K01 (1 x 96 wells)

PRT31-K02 (2 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0

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INTENDED USE

The Eagle Biosciences Human Proteinase 3 ELISA Kit is to be used for the quantitative determination of Proteinase 3 in serum and plasma samples. The Eagle Biosciences Human Proteinase 3 ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

INTRODUCTION

Proteinase 3 (PR3) is a major antigen recognized by autoantibodies directed against cytoplasmic proteins of neutrophilic granulocytes and monocytes (so called anti-neutrophil cytoplasmic autoantibodies (ANCA)). ANCA are able to activate primed neutrophils to produce oxygen radicals and release lytic enzymes, including PR3. PR3 was identified as the target antigen of ANCA in Wegener's granulomatosis (WG). ANCA directed against PR3 (PR3-ANCA) can interfere with the binding of PR3 to its physiological inhibitor alpha1-antitrypsin (alpha1-AT) and with the proteolytic activity of PR3. At the site of inflammation PR3 can cleave the complex between these inhibiting ANCA and PR3 itself, leaving active PR3. Autoantibodies to PR3 are potent activators of the 5-lipoxygenase pathway in primed human neutrophils. Extracellular free arachidonic acid, as present at an inflammatory focus, synergizes with such autoantibodies to evoke full-blown lipid mediator generation, granule secretion and respiratory burst.

PR3 is a neutral serine proteinase, which is localized in the azurophilic granules of neutrophils and in granules of monocytes and can be detected in the membrane of secretory vesicles. PR3 degrades a number of extracellular matrix proteins such as elastin and inactivates human C1 inhibitor. Membrane-associated PR3 is also able to activate caspase-3 without triggering apoptosis of neutrophils, which is possibly a neutrophil survival mechanism. In addition, PR3 is involved in myeloid differentiation and is, therefore, also called myeloblastin. PR3 matures/activates major inflammatory cytokines like membrane bound TNF, IL1beta and IL18. It has an important role in inflammasome-independent induction of inflammation and an important role in inflammatory diseases as Rheumatoid Arthritis, IBD and inflammatory-associated disease as Type 2 diabetes, cardiovascular diseases and arteriosclerosis.

PRINCIPLE OF THE ASSAY

The Human Proteinase 3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours. The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay. Samples and standards are incubated in microtiter wells coated with antibodies recognizing Human Proteinase 3. Biotinylated tracer antibody will bind to the captured Human Proteinase 3. Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody. Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB). The enzyme reaction is stopped by the addition of oxalic acid. The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the Human Proteinase 3 standards (log). The Human Proteinase 3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT FEATURES

- Working time of 3 ½ hours.
- Minimum concentration which can be measured is 0.63 ng/ml.
- Measurable concentration range of 0.63 to 40 ng/ml.
- Working volume of 100 µl/well.



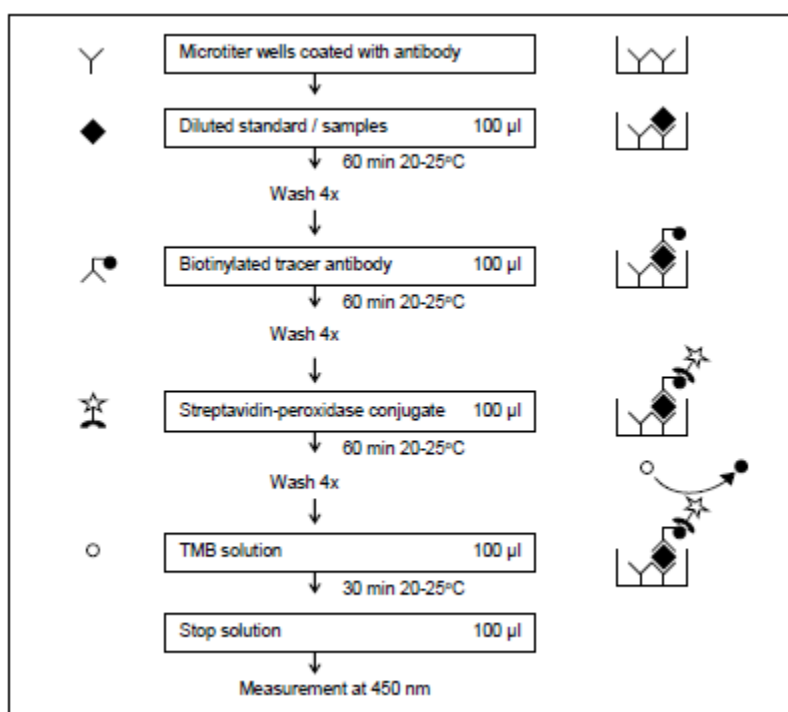
CROSS REACTIVITY

Potential cross-reacting proteins detected in the human Proteinase 3 ELISA:

Cross reactant	Reactivity
Mouse	No
Rat	No
Pig	No
Human Elastase	No
Human alpha-1 antitrypsin	No

Cross-reactivity for other species or proteins/peptides has not been tested.

PROTOCOL OVERVIEW



- The Human Proteinase 3 ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- The efficient format of a plate with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing Human Proteinase 3.
- Biotinylated tracer antibody will bind to the captured Human Proteinase 3.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.



- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the Human Proteinase 3 standards (log).
- The Human Proteinase 3 concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

KIT COMPONENTS AND STORAGE INSTRUCTIONS

Kit Component	Quantity PRT31-K01	Quantity PRT31-K02	Color Code
Wash buffer 20x	1 vial (60 ml)	1 vial (60 ml)	Colorless
Dilution Buffer 10x	1 vial (15 ml)	1 vial (15 ml)	Green
Sample dilution buffer 10x	1 vial (15 ml)	1 vial (15 ml)	Blue
Standard	2 vials, lyophilized	4 vials, lyophilized	White
Tracer, biotinylated	1 vial, 1 ml lyophilized	2 vials, 1 ml lyophilized	White
Streptavidin-peroxidase	1 tube, 0.25 ml in solution	1 tube, 0.25 ml in solution	Brown
TMB substrate	1 vial (22 ml)	1 vial (22 ml)	Brown
Stop solution	1 vial (22 ml)	1 vial (22 ml)	Red
12 Microtiter strips, pre-coated	1 plate	2 plates	
Certificate of Analysis	1	1	
Manual	1	1	
Data collection sheet	2	2	

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard and conjugate in lyophilized form and the streptavidin-peroxidase in concentrated solution are stable until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact amount of standard is indicated on the label of the vial and the Certificate of Analysis.
- The standard is single use. After reconstitution the standard cannot be stored.
- Once reconstituted the tracer is stable for 1 month if stored at 2 - 8°C.
- The streptavidin-peroxidase can only be stored in concentrated solution and is not stable when stored diluted.
- Upon receipt, foil pouch around the plates should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed for 1 month if stored at 2 - 8°C.

MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated micropipettes and disposable tips.
- Distilled or de-ionized water.
- Plate washer: automatic or manual.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.
- Adhesive covers.
- Centrifuge for 1 ml tubes.



WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Open vials carefully: vials are under vacuum.
- It is advised to spin down streptavidin-peroxidase tubes before use.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse micro wells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.
- The standard is of human origin. It was tested for various viruses and found negative. Since no test method can offer complete assurance that infectious agents are absent, this reagent should be handled as any potentially infectious human serum or blood specimen. Handle all materials in contact with this reagent according to guide-lines for prevention of transmission of blood-borne infections.

SAMPLE PREPARATION

Collection and handling

Serum or plasma

Collect blood using normal aseptic techniques. Blood samples should be kept on ice. If serum is used, separate serum from blood after clotting at room temperature within one hour by centrifugation (1,500xg at 4°C for 15 min). Transfer the serum to a fresh polypropylene tube. If plasma is used, separate plasma from blood within 20 minutes after blood sampling by centrifugation (1,500xg at 4°C for 15 min). Transfer the plasma to a fresh polypropylene tube.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of Human Proteinase 3. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of Human Proteinase 3 activity and give



erroneous results. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples. Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Dilution procedures

Serum or plasma samples

Human Proteinase 3 can be measured accurately if serum or plasma samples are diluted at least 5x with supplied sample dilution buffer in polypropylene tubes. Optimal dilution is dependent on sample quality and expected PR3 quantity and should be determined for the specific sample set/study.

Comment regarding recommended sample dilution

The mentioned dilution for samples is a minimum dilution and should be used as a guideline. The recovery of Human Proteinase 3 from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of Human Proteinase 3.

Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

Guideline for dilution of samples

Please see the table below for recommended sample dilutions. Volumes are based on a total volume of at least 230 µl of diluted sample, which is sufficient for one sample in duplicate in the ELISA. For dilution of samples we recommend to use at least 10 µl of sample.

Dilution	Pre-dilution	Amount of sample or pre-dilution required	Amount of Dilution buffer required
10x	Not necessary	25 µl (sample)	225 µl
20x	Not necessary	15 µl (sample)	285 µl
50x	Not necessary	10 µl (sample)	490 µl
100x	Not necessary	10 µl (sample)	990 µl
500x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	490 µl
1000x	Recommended: 10x (see nr.1)	10 µl (pre-dilution)	990 µl
2000x	Recommended: 20x (see nr. 2)	10 µl (pre-dilution)	990 µl
5000x	Recommended: 50x (see nr.3)	10 µl (pre-dilution)	990 µl

REAGENT PREPARATION

Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.

Wash buffer

Prepare wash buffer by mixing 60 ml of 20x wash buffer with 1140 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20x wash buffer with 19 parts of distilled or de-ionized water.



Dilution buffer

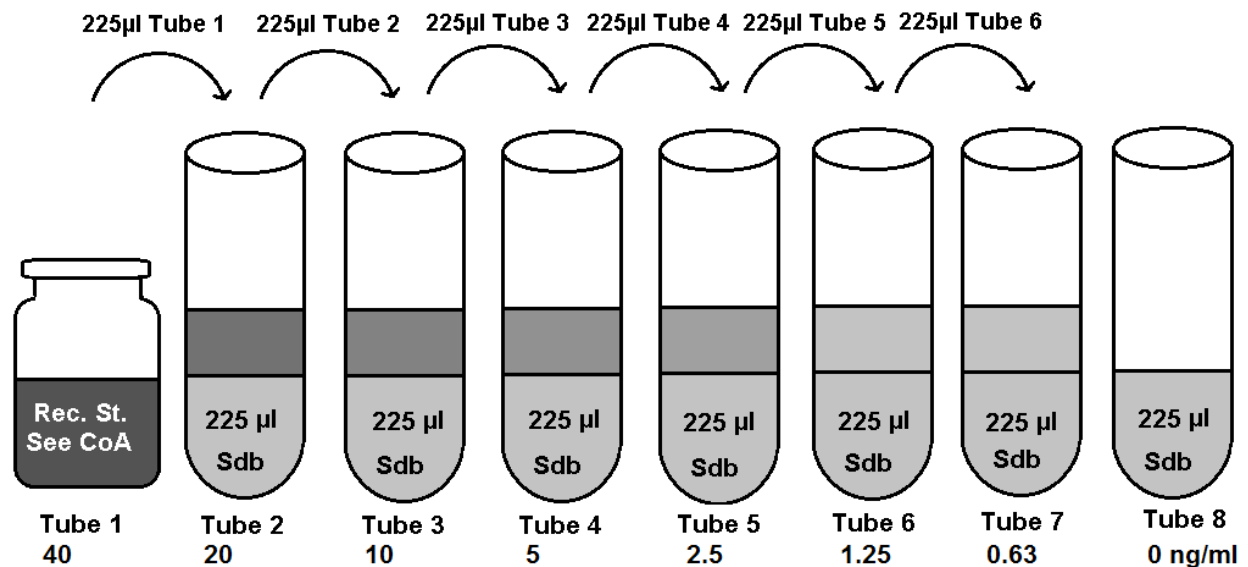
Prepare dilution buffer by mixing 15 ml of the 10x dilution buffer with 135 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of dilution buffer by diluting 1 part of the 10x dilution buffer with 9 parts of distilled or de-ionized water. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within one hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.

Sample Dilution buffer

Prepare sample dilution buffer by mixing 15 ml of the 10x sample dilution buffer with 135 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. In case less volume is required, prepare the desired volume of sample dilution buffer by diluting 1 part of the 10x sample dilution buffer with 9 parts of distilled or de-ionized water. Concentrated sample dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within one hour, concentrated sample dilution buffer can be warmed up to 37°C. Do not shake the solution.

Standard solution

The standard is reconstituted by pipetting the amount of sample dilution buffer mentioned on the CoA in the standard vial. Use the standard vial as Tube 1 in Figure 1. Prepare each Human Proteinase 3 standard in polypropylene tubes by serial dilution of the reconstituted standard with sample dilution buffer as shown in Figure 1*. After reconstitution the standard cannot be stored for repeated use.



Tracer solution

The tracer is reconstituted by pipetting 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml tracer with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. In case less volume is required, prepare the desired volume of tracer by diluting 1 part of the reconstituted tracer with 11 parts of dilution buffer.



Streptavidin-peroxidase solution

It is advised to spin down streptavidin-peroxidase tubes before use. Prepare the streptavidin-peroxidase solution by mixing 0.25 ml of the 100x streptavidin-peroxidase solution with 24.75 ml dilution buffer, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of streptavidin-peroxidase solution by diluting 1 part of the 100x streptavidin-peroxidase solution with 99 parts of dilution buffer.

ELISA PROTOCOL

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Cover the tray and tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer using a plate washer or as follows*:
 - a. Carefully remove the cover, avoid splashing.
 - b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
 - c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
 - d. Repeat the washing procedure 5b/5c three times.
 - e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted biotinylated tracer to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray and incubate the tray for 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray and incubate the tray for 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
13. Cover the tray and incubate the tray for 30 minutes at room temperature. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

*) In case plate washer is used, please note: use of a plate washer can result in higher background and decrease in sensitivity. We advise validation of the plate washer with the manual procedure.

Make sure the plate washer is used as specified for the manual method.



INTERPRETATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, control and samples.
- If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
- The mean absorbance of the zero standard should be less than 0.3.
- Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale).
- If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

TECHNICAL HINTS

- User should be trained and familiar with ELISA assays and test procedure.
- If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
- Since exact conditions may vary from assay to assay, a standard curve must be established for every run. Samples should be referred to the standard curve prepared on the same plate.
- Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
- Each time the kit is used, fresh dilutions of standard, sample, tracer, streptavidin-peroxidase and buffers should be made.
- Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
- To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- The waste disposal should be performed according to your laboratory regulations.

PERFORMANCE CHARACTERISTICS

Linearity

The linearity of the assay was determined by serially diluting 10 EDTA plasma samples containing human Proteinase 3. The diluted sample was measured in the assay. Samples were diluted at least 5 times.

EDTA plasma samples	Conc. Ng/ml	CV (%)
Sample 1	21	21.7
Sample 2	66	11.4
Sample 3	40	14.3
Sample 4	41	21.9
Sample 5	21	15.1



Sample 6	58	6.2
Sample 7	21	16.6
Sample 8	23	11.2
Sample 9	31	4.8
Sample 10	33	7.6

Recovery

Normal human blood samples (plasma) containing baseline levels of human Proteinase 3, were spiked with human Proteinase 3, in concentrations of 20, 5 and 1.25 ng/ml. Samples with and without human Proteinase 3, were incubated for 30 minutes at room temperature. Samples were measured using the ELISA. Values for human Proteinase 3, ranged between 92% and 113%.

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.