Human Neutrophil Elastase Detection ELISA Kit

Catalog # 6057

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify human neutrophil elastase
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	1000 - 16 pg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Liquid samples and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (3.0-9.7%)/Inter-Assay (2.9-8.0%)/Spiking Test (94-95%)
NOTES:	N/A

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INTRODUCTION

Neutrophils are frontline innate immune cells that defend the host from bacterial and viral infection through phagocytosis and the activity of intracellular antimicrobial proteins. Under certain pathological conditions or following *in-vitro* stimulation, neutrophils can release extracellular, web-like structures termed neutrophil extracellular traps (NETs). NETs are composed of chromatin decorated with DNA and a variety of neutrophil proteins, including neutrophil elastase (NE), myeloperoxidase (MPO), cathepsin G, and the S100A8/A9 (calprotectin) complex (1-3).

Neutrophil elastase is a serine protease stored in azurophilic (primary) granules and released upon neutrophil activation and degranulation. NE is a single-chain protein with a molecular weight of approximately 29 kDa and functions in extracellular matrix degradation, cytokine processing, and modulation of immune responses. Because of these activities, dysregulated NE contributes to the pathogenesis of acute lung injury, chronic obstructive pulmonary disease (COPD), cystic fibrosis, rheumatoid arthritis, sepsis, and ANCA-associated vasculitis (4,5).

For *in-vitro* studies of neutrophil activation and NET release, enzymatic activity assays (for example, NE and MPO activity) provide functional information about enzyme release and activation. Quantification of NE and MPO in cell culture medium by ELISA is widely used as a biochemical marker of NET release. Typical cell sources for NET assays include primary human neutrophils isolated from whole blood and established cell lines (e.g., HL-60 cells) (6).

Chondrex, Inc. provides a Human Neutrophil Elastase Detection ELISA Kit (Cat # 6057) suitable for assaying cell culture supernatant, biological fluid, serum, and plasma, as well as a Mouse MPO Detection ELISA Kit (Cat # 6051) that cross-reacts with human MPO. These products can be used to study NET release dynamics under neutrophil activation in experimental systems.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Standard Human Neutrophil Elastase (60571)	1 vial	1000 pg, lyophilized	-20°C
Anti-Human Neutrophil Elastase Detection Antibody (60573)	1 vial	100 µl	-20°C
Solution C - Sample/Standard/Detection Antibody Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-20°C
Chromogen Dilution Buffer (90022)	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid (9016)	1 bottle	10 ml	-20°C
Wash Buffer, 20X (9005)	1 bottle	50 ml	-20°C
Anti-Human Neutrophil Elastase Antibody Coated ELISA Plate (Brown)	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of diluted standards and samples into wells

↓ Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted Detection Antibody

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution

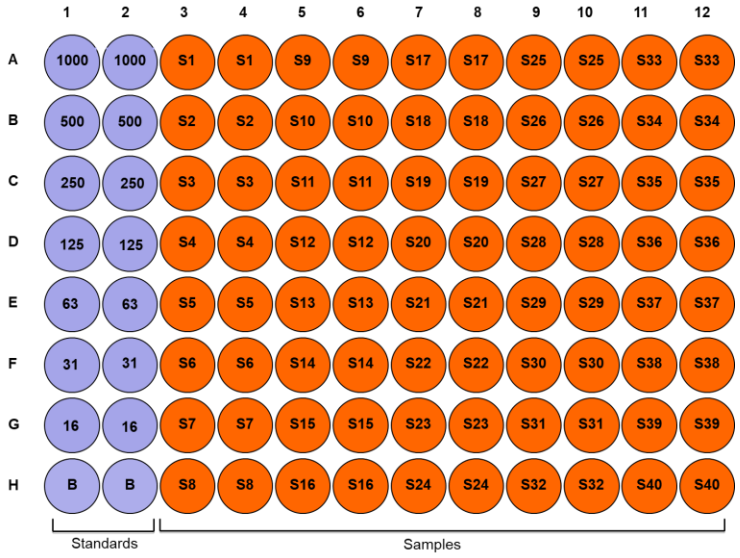
↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING



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NOTES BEFORE USING ASSAY

NOTE 1: It is recommended that the standard and samples be run in duplicate.

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

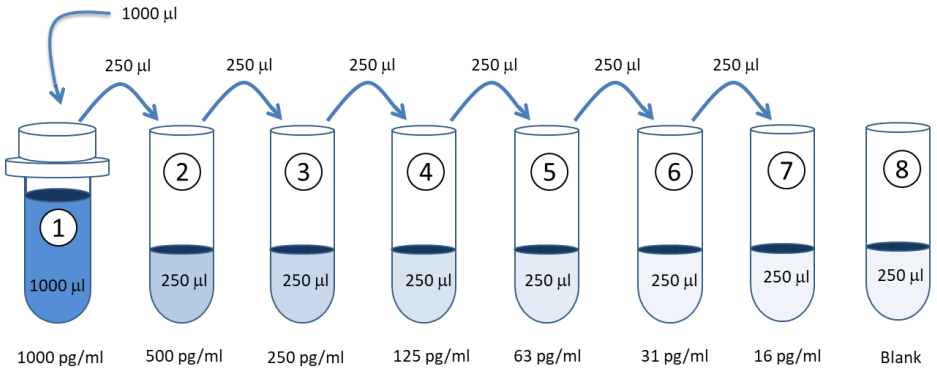
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 1000 - 16 pg/ml. Dissolve one vial of Standard (1000 pg/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution C) and keep it as standard stock. Then serially dilute it with Solution C. For example, mix 250 µl of the 1000 pg/ml solution with an equal volume of Solution C to make a 500 pg/ml solution, and then repeat it five more times for 250, 125, 63, 31, and 16 pg/ml standard solutions.



2. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution C depending on the estimated human neutrophil elastase levels in the samples. Two or three different sample dilutions are recommended if the human neutrophil elastase levels in the samples are unknown.

3. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

4. **Wash**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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5. **Add Detection Antibody**: Dilute one vial of detection antibody in 10 ml Solution C. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 1 hour.

Strip #	Detection Antibody (µl)	Solution C (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Solution D as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

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CALCULATING RESULTS

1. Average the duplicate OD values for the standards, blanks (B), and test samples.

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of the standards against the pg/ml of human neutrophil elastase standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 16 to 1000 pg/ml.

4. The pg/ml of human neutrophil elastase in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the human neutrophil elastase concentration (pg/ml) in the original test samples.

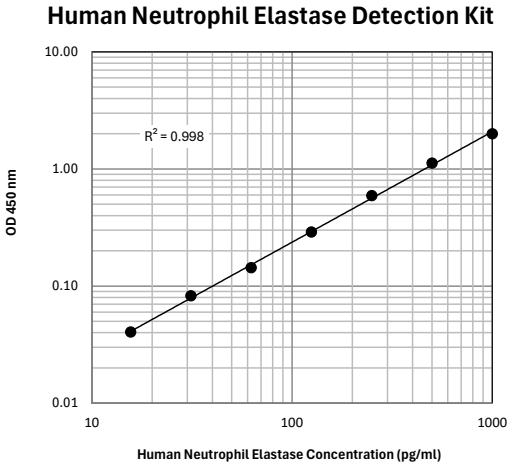

Figure 1 - A Typical Standard Curve for the Human Neutrophil Elastase Detection ELISA Kit

VALIDATION DATA

Table 1 - Reproducibility Data for the Human Neutrophil Elastase Detection ELISA Kit

Test	31 pg/ml	125 pg/ml	500 pg/ml
Intra-Assay CV (%)	9.7	6.7	3.0
Inter-Assay CV (%)	6.3	8.0	2.9
Spike Test* (%)	95%	94%	95%

*Known amounts of human neutrophil elastase were added to samples and then diluted with Sample/Standard Dilution Buffer (Solution C) to assay human neutrophil elastase by ELISA.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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