ELA2 (Human) ELISA Kit

Catalog Number IRKTAH2518

96 assays

Version: 04

Intended for research use only

Table of Contents

Introduction

Background

Human Neutrophil Elastase (NE), also known as ELA2, is a subfamily of serine proteinase elastases. It consists of 218 amino acid residues with a molecular weight of 23 kDa and two asparagine-linked carbohydrate side chains (1). NE is stored in neutrophil lysosomes azurophil granules during neutrophil differentiation, and is involved in a variety of immune defense reactions and degenerative and inflammatory diseases (2-3). Upon infection, the activated neutrophils release NE which then hydrolyzes azurophil granule proteins and extracellular matrix proteins: elastin, collagen, and proteoglycan (4). As a host defense protein, NE degrades bacterial outer membrane and virulence proteins (5-6). When expressed abnormally, NE causes the development of pulmonary emphysema (7). NE mutations are associated with cyclic neutropenia and severe congenital neutropenia (8-9).

Principle of the Assay

The ELA2 (Human) ELISA Kit is designed for detection of human Neutrophil Elastase in plasma, serum, urine, saliva, milk, and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures human Elastase in less than 4 hours. A polyclonal antibody specific for human Elastase has been pre-coated onto a 96-well microplate with removable strips. Elastase in standards and samples is sandwiched by the immobilized antibody and the biotinylated polyclonal antibody specific for Elastase, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

General Information

Materials Supplied

List of component

Component	Amount
Human Neutrophil Elastase Microplate: A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Neutrophil Elastase.	1
Sealing Tapes: pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3
Human Neutrophil Elastase Standard: Human Neutrophil Elastase in a buffered protein base	20 ng, lyophilized
Biotinylated Neutrophil Elastase Antibody (80x): A 80-fold concentrated biotinylated polyclonal antibody against Neutrophil Elastase	100 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 , 2 bottles
Streptavidin-Peroxidase Conjugate (SP Conjugate): A 100-fold concentrate	80 µl
Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine	8 ml
Stop Solution: A 0.5 N hydroxychloric acid to stop the chromogen substrate reaction	12 ml

Storage Instruction

- ✓ Store components of the kit at 2-8℃ or -20℃ upon arrival up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20℃.
- ✓ Store Microplate, Diluent Concentrate (10×), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8℃.
- ✓ Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- ✓ Diluent (1x) may be stored for up to 1 month at 2-8℃.
- ✓ Store Standard at 2-8℃ before reconstituting with Diluent and at -20℃ after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ 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

Precautions for Use

- Precautions

✓ Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-antibody, and SP 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 protocol. However, the user should determine the optimal dilution factor.

✓ Spin down the SP conjugate vial and the biotinylated-antibody vial before opening and using contents.

✓ This kit is for research use only.

✓ The kit should not be used beyond the expiration date.

✓ The Stop Solution is an acid solution

Assay Protocol

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8℃.
- Standard Curve: Reconstitute the 20 ng of Neutrophil Elastase Standard with 2 ml of EIA Diluent to generate a solution of 10 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (10 ng/ml) 1:2 with EIA Diluent to produce 5, 2.5, 1.25, 0.625, 0.313, and 0.156 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20℃.

Standard Point	Dilution	[Elastase] (ng/ml)
P1	Standard (10 ng/ml)	10.000
P2	1 part P1 + 1 part EIA Diluent	5.000
P3	1 part P2 + 1 part EIA Diluent	2.500
P4	1 part P3 + 1 part EIA Diluent	1.250
P5	1 part P4 + 1 part EIA Diluent	0.625
P6	1 part P5 + 1 part EIA Diluent	0.313
P7	1 part P6 + 1 part EIA Diluent	0.156
P8	EIA Diluent	0.000

- Biotin Elastase Antibody (80x): Spin down the antibody briefly and dilute the desired amount of the antibody 1:80 with EIA Diluent. Any remaining solution should be frozen at -20℃.
- Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20℃.

Sample Preparation

- Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 xg for 10 minutes and assay. Dilute samples 1:50 into EIA Diluent. 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 anticoagulant.)
- Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 xg for 10 minutes. Remove serum and assay. Dilute samples 1:50 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- Cell Culture Supernatants: Centrifuge cell culture media at 2000 xg for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20°C or below. Avoid repeated freeze-thaw cycles.
- Urine: Collect urine using sample pot. Centrifuge samples at 600 xg for 10 minutes. Dilute Urine 1:8 with EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Saliva: Collect saliva using sample tube. Centrifuge samples at 600 xg for 10 minutes. Dilute Saliva 1:1000 with EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- Milk: Collect milk using sample tube. Centrifuge samples at 600 xg for 10 minutes. Dilute Milk 1:200000 with EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30℃).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Neutrophil Elastase Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
4. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
5. Add 50 µL of Biotinylated Neutrophil Elastase Antibody to each well and incubate for one hour.
6. Wash the microplate as described above.
7. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash the microplate as described above.
9. Add 50 µL of Chromogen Substrate per well and incubate for about 8 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
11. 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

Calculation of Results

- ✓ 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 four-parameter or log-log logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor

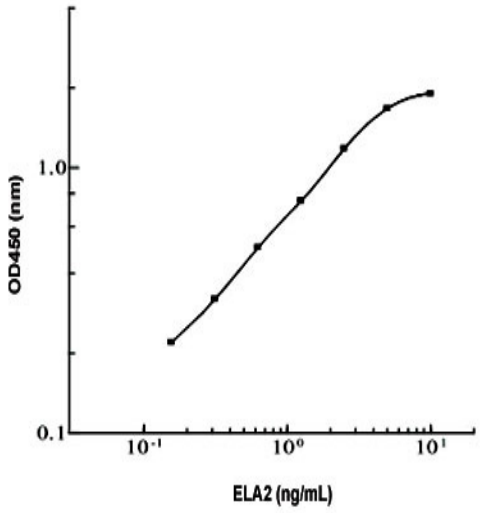

Figure 1: Typical Standard Curve for ELA2 (Human) ELISA Kit

Performance Characteristics

- ✓ The minimum detectable dose of Elastase is typically ~0.15 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 5.1% and 7.4% respectively.

- Linearity

	Average Percentage of Expected Value	
Sample Dilution	Plasma	Serum
1:25	88%	92%
1:50	95%	101%
1:100	101%	109%

	Average Percentage of Expected Value
Sample Dilution	Saliva
1:500	91%
1:1000	97%
1:2000	103%

	Average Percentage of Expected Value
Sample Dilution	Urine
1:4	89%
1:8	98%
1:16	101%

- Recovery

Standard Added Value	0.5 – 5 ng/mL
Recovery %	84 - 104 %
Average Recovery %	96%

- Cross-Reactivity

Species	% Cross Reactivity
Canine	25%
Bovine	None
Monkey	5%
Mouse	1%
Rat	None
Swine	1%
Rabbit	None

Resources

Plate Layout

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
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12								