Quantikine® ELISA

Mouse Neutrophil Elastase/ELA2 Immunoassay

Catalog Number MELA20

For the quantitative determination of mouse Neutrophil Elastase (ELA2) concentrations in cell culture supernates, tissue lysates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Neutrophil Elastase (ELA2), also known as polymorphonuclear leukocyte elastase, is a serine protease belonging to the chymotrypsin family. Located primarily in the azurophil granules of polymorphonuclear leukocytes, ELA2 function is the degradation of many extracellular matrix macromolecules (1, 2).

A-1 Antitrypsin (Serpin A1) and secretory leukocyte protease inhibitor (SLPI) have been shown to inhibit ELA2 activity (3). This protein may be involved in lung emphysema, cystic fibrosis, the adult respiratory distress syndrome (ARDS), rheumatoid arthritis, tumor invasion and infectious diseases (1).

ELA2 may have a potential therapeutic application for treating cardiovascular disease (4). Sepsis caused high levels of ELA2 in mouse lung homogenates and indicated that aerobic exercise in later life may reduce severity of a septic response(5).

The Quantikine® Mouse Neutrophil Elastase/ELA2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure ELA2 in cell culture supernates, tissue lysates, serum, and plasma. It contains NS0-expressed recombinant mouse ELA2 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant mouse ELA2. Results obtained using natural mouse ELA2 showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse ELA2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse ELA2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any ELA2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse ELA2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of ELA2 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse ELA2 Microplate	894847	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse ELA2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse ELA2 Standard	894849	2 vials of recombinant mouse ELA2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a fresh standard and control for each assay. Discard after use.
Mouse ELA2 Control	894850	2 vials of recombinant mouse ELA2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse ELA2 Conjugate	894848	12 mL/vial of a polyclonal antibody specific for mouse ELA2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-21	895215	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5P	895151	21 mL/vial of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	Adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene tubes** for dilution of standards and controls.

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347), Lysis Buffer 17 (R&D Systems, Catalog # 895943), or RIPA Buffer with Protease inhibitors.
- PBS

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates - Lysates were prepared prior to assay as described in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution due to high endogenous levels. A suggested 10-fold dilution is 15 µL of sample + 135 µL of Calibrator Diluent RD5P (diluted 1:5)*.

Tissue lysates utilizing a RIPA Buffer require an initial 4-fold dilution due to a matrix effect. The suggested range for total tissue lysate protein added is 2-250 µg/well.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse ELA2 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Assay the control undiluted. Mix thoroughly.

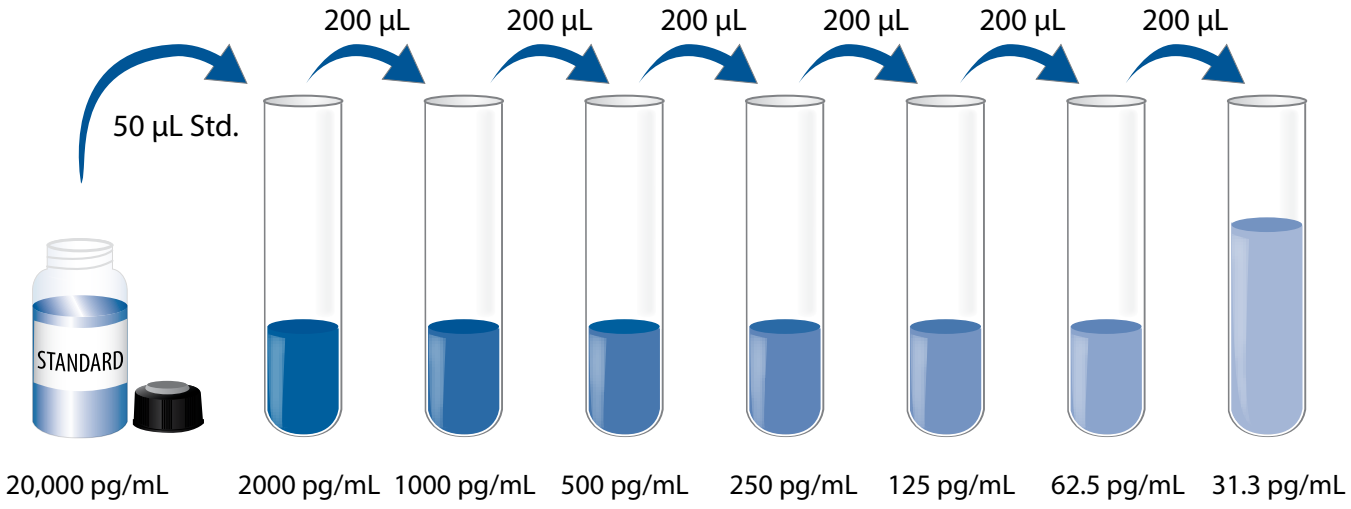
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 10 mL of Calibrator Diluent RD5P to 40 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:5).

Mouse ELA2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse ELA2 Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse ELA2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on a shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Sample may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

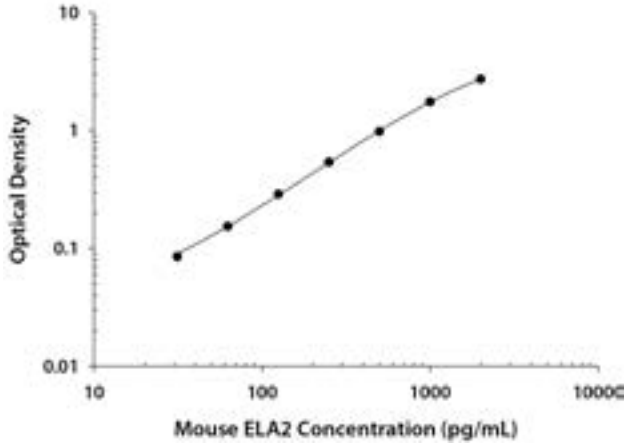
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse ELA2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.022	0.022	—
31.3	0.083 0.086	0.085	0.063
62.5	0.152 0.156	0.154	0.132
125	0.286 0.291	0.289	0.267
250	0.530 0.549	0.540	0.518
500	0.978 0.984	0.981	0.959
1000	1.727 1.766	1.747	1.725
2000	2.735 2.741	2.738	2.716

PRECISION

Intra-Assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	104	254	735	96.9	238	709
Standard deviation	1.28	3.64	15.7	9.39	17.4	43.6
CV (%)	1.2	1.4	2.1	9.7	7.3	6.1

RECOVERY

The recovery of mouse ELA2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	90-102%
Serum* (n=4)	112	104-122%
EDTA plasma* (n=4)	108	95-125%
Heparin plasma* (n=4)	108	101-118%
Lysis buffer (n=3)	96	91-97%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse ELA2 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Tissue lysates (n=3)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	104	102	103	104	106
	Range (%)	97-113	98-107	101-104	103-105	103-109
1:4	Average % of Expected	106	101	108	105	111
	Range (%)	99-120	95-106	104-112	102-108	107-118
1:8	Average % of Expected	107	102	110	108	110
	Range (%)	99-124	100-105	106-113	104-111	107-117
1:16	Average % of Expected	107	100	109	107	104
	Range (%)	99-126	99-101	105-113	102-111	101-110

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of mouse ELA2 ranged from 0.574-3.37 pg/mL. The mean MDD was 1.60 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse ELA2 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse ELA2 in this assay.

	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	5576	2988-12,277	2830
EDTA plasma (n=5)	2963	2065-3749	612
Heparin plasma (n=5)	3251	2745-3819	494

Cell Culture Supernates:
Mouse bone marrow cells were cultured in RPMI 1640 and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 25 ng/mL of recombinant mouse SCF, and 50 ng/mL of recombinant mouse IFN-γ for 7 days. 50 mL of the cultured volume was removed and 50 mL of fresh media was added and grown for an additional 3 days. An aliquot of the cell culture supernate was removed, assayed for mouse ELA2, and measured 618 pg/mL.

Mouse lung and spleen tissues were processed separately. Tissues were rinsed with PBS and placed on ice. Tissue was homogenized and cultured in RPMI 1640 and supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cell were cultured unstimulated for 3 days. Aliquots of the cell culture supernates were removed, assayed for levels of mouse ELA2, and measured 149 pg/mL and 712 pg/mL, respectively.

Tissue Lysates - Mouse lung and spleen tissue were process separately. Tissues were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. Cell Lysis Buffer 2 was added at 1:1 ratio. Tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Whole cell extract protein concentration was qualified using a total protein assay and assayed for mouse ELA2.

Tissue lysate	(µg/well)	(pg/mL)
Lung	50	763
Spleen	25	1862

SPECIFICITY

This assay recognizes natural and recombinant mouse ELA2

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse ELA2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

MMP-2
Serpin A1a
Serpin A1c
SLPI

Recombinant human:

ELA2
Myeloblastin
Trappin-2

REFERENCES

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