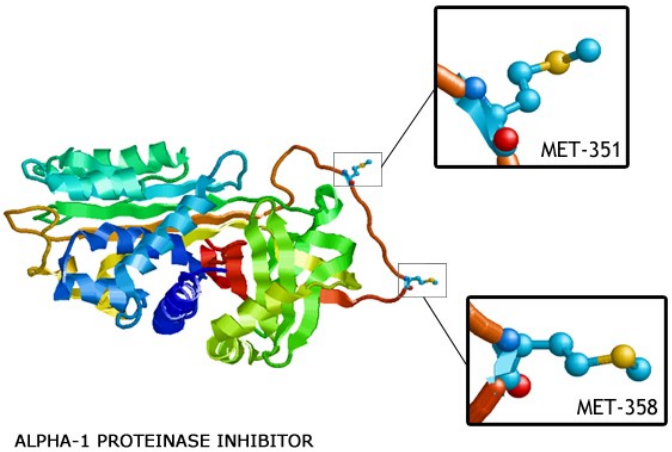
Alpha-1 Proteinase Inhibitor (A1PI) Activity Assay

Product NWK-A1PI01

For Research Use Only



Assay system for measuring the activity of Alpha-1 Proteinase Inhibitor (A1PI) also known as Alpha-1 Antitrypsin (A1AT) and Alpha-1 Antiproteinase (A1AP)

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Introduction:

Alpha-1 proteinase inhibitor (A1PI), also known as alpha-1 antitrypsin (A1AT) and alpha-1 antiproteinase (A1AP) is the archetypal member of superfamily serine protease inhibitors (SERPINs). A1PI is the only known inhibitor of elastase but also inhibits trypsin and chymotrypsin. As an acute phase protein, A1PI is commonly associated with regulation of inflammatory reactions, especially in lung tissue where its main function is to inhibit neutrophil elastase. Binding of the target protease to the reactive center loop (RCL) of A1PI is irreversible and proteolysis results in a conformational change rendering A1PI inactive. Another notable feature of A1PI is that its activity can be regulated by oxidation of the sulfur containing amino acid methionine. Reactive oxygen and nitrogen species (ROS & NOS) can both react with A1P to create methionine sulfoxide (MetO) residues at key sites resulting in inactivation of the enzyme. Regulation of A1PI in this fashion is reversible by the action of methionine sulfoxide reductase, an important regulatory enzyme of interest in redox related research. The action of ROS and NOS in helping to regulate the activity of cellular housekeeping proteins such as A1PI is fast becoming a major area of interest in researching many disease states. In contrast to ELISA measures of total protein, monitoring of A1PI activity levels can provide useful information about the effect of ROS and NOS on actual enzyme function. Measurement of A1PI activity in both control and research samples can be useful as an index of oxidative stress effect on proteins.

Intended Use:

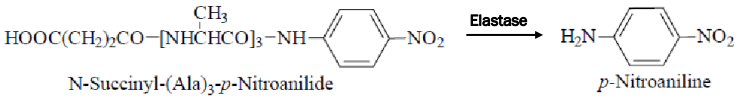
NWK-A1PI01 is intended for measuring the activity of alpha-1 proteinase inhibitor in biological research samples.

Test Principle:

Alpha-1 proteinase inhibitor rapidly, specifically and irreversibly binds to the active site of elastase. The molar reaction ratio is 1:1 such that measuring inhibition of elastase activity becomes a convenient method for quantifying A1PI activity. In *Phase One* of this assay a known quantity of elastase is allowed to react with sample A1PI. Elastase Reagent is provided in excess such that the remaining elastase activity can be measured as a means of determining the level of A1PI mediated inhibition.

$$[\text{Elastase}]_{\text{Known}} + [\text{A1PI}]_{\text{Sample}} = [\text{Elastase/A1P1}]_{\text{Complex}} + [\text{Elastase}]_{\text{Test}}$$

In *Phase 2* of the assay N-succinyl-$(Ala)_3$-*p*-nitroanilide (NSAN) reacts with the remaining Elastase to cleave p-nitroanaline with a maximal absorbance at 410 nm.



Test Principle (continued):
Remaining sample elastase is determined by comparing sample A_{410} to a five point standard curve generated at the time of sample testing. The amount of elastase ***i****nhibition* is found by subtracting the remaining ***t****est* sample elastase (measured) from the ***k****nown* elastase quantity (as shown on the vial).

$$[E_i] = [E_k] - [E_t]$$

Since A1PI and elastase react in a one to one molar ratio, the concentration of A1PI in the reaction mix is the same as the calculated elastase inhibition.

$$[A1PI_{Rxm}] = [E_i]$$

A1PI concentration in the original **s**ample is therefore calculated as:

$$[A1PI_s] = [E_i] \ * \text{Sample Dilution}$$

General Specifications:

Format:	96 well colorimetric
Number of tests:	Triplicate = 27 Duplicate= 43
Specificity:	Alpha-1 Proteinase Inhibitor (A1PI)
Sensitivity:	50 nM Elastase or nM A1PI equivalents.

*Effective Range: 50 - 300 nM Residual Elastase or nM A1PI equivalents.
* Note: Measured values <50 nM are not valid and a less dilute sample should be tested. Samples >300 nM A1PI should be retested at a greater dilution.

Kit Contents

Elastase Calibrator (~600 nM in Assay Buffer):	1 X 0.75 mL
Elastase Reagent (~300 nM in Assay Buffer):	1 X 5 mL
NSAN Reagent (600 µM in Assay Buffer)	1 X 5 mL
Assay Buffer: (Containing Tris-HCl, pH 8.0)	1 X 100 mL
Microplate:	1 X 96 wells

Required Materials Not Provided:
Multichannel or repeater pipettes capable of precisely delivering 50 µL.
Pipette capable of precisely delivering 100 – 1000 µL.
Glass or plastic tubes for sample dilutions.
Glacial Acetic Acid optional…if stopping the reaction is desired in order to stabilize the color for later reading (stable for several hours after stopping).

Required Instrumentation:
Microtiter plate reader with 405 nm* capability.
**Note: Maximal absorbance for p-nitroaniline of interest is 410 nm.*

Warnings, Limitations, Precautions:
Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage Instructions:
Store all components at 4°C until immediately before use. Do not freeze.

Assay Preparation
1. Determine the number of wells required to assay calibrators, samples and controls for the appropriate number of replicates.

2. Create an assay template showing positioning of calibrators, samples and controls.

Calibrator Preparation:
1. Label 6 vials as 300, 150, 75, 37.5, 18.75 and 0 nM Elastase.

2. Add 250 µL Assay Buffer to each vial.

3. Add 250 µL of 600 nM Elastase Standard to 300 nM vial, mix well, then continue with serial dilution down to 18.75 nM. Leave the 0 nM vial with assay buffer only.

Reagent Preparation:
All reagents other than the calibrator are provided ready to use.

Sample Handling/Preparation
Plasma
Collect blood and harvest the plasma fraction. Store plasma cold for testing on day of draw. If testing on a different day, samples may be stored short term (1-2 days) at -20°C or -70°C for longer term storage. Samples should be diluted 1:150 in Assay Dilution Buffer just prior to assay. Plasma levels of A1PI in healthy humans ranges from 20 - 50 µM.

Tissue:
Less is known about the existence of A1PI levels in various tissues. While it may be possible to test A1PI in tissue samples such as lung homogenate, it is beyond the scope of this product insert to advise on sample handling and dilution schemes for potential tissue homogenates.

Assay Protocol:

1. Add 50 µL **Assay Buffer** to designated calibrator wells

2. Add 50 µL **Diluted Calibrator** to designated calibrator wells.

3.. Add 50 µL **Diluted Sample** to designated test sample wells.

4. Add 50 µL **Elastase Reagent** to designated test sample wells.*
*Note: Do not add Elastase Reagent to calibrator wells.

5. Incubate for 45 minutes at room temperature in humid, closed container.

6. Add 50 µL **NSAN Reagent** to all wells.

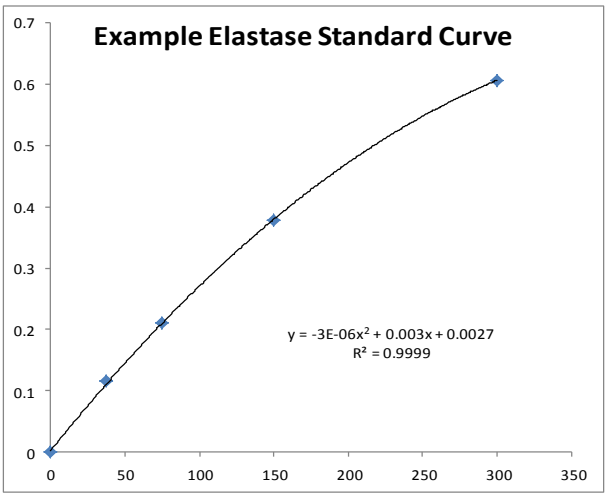
7. Incubate for 15 minutes.

8. Optional Step: Add 10 µL **Stop Solution** and shake to mix. If not opting to use stop solution, proceed to step 9.

9. Record the absorbance at 410 nm (405 is acceptable).

Data Analysis (Elastase Calibration Method):

Plot the elastase calibrator concentration vs. 410 nm absorbance. For best results we recommend using the 2nd order polynomial (quadratic) curve fit for data analysis.



Example Data & Calculation

Elastase Reagent Concentration: 0.300 µM... **Note: This Value is Lot Specific**			
	Rep 1	Rep 2	Average
Original Elastase Concentration	300 nM	300 nM	300 nM
Measured Sample Elastase (1/150 Plasma)	157.51 nM	159.46 nM	158.49 nM
A1PI in Reaction Mix ($A1PI_{Rxm}$)	142.49 nM	140.54 nM	141.51 nM
A1PI in Original Sample ($A1PI_{Rxm}$ X 150)	21.374 µM	21.081 µM	21.227 µM

Performance Details:

Precision

Inter and Intra assay precision was estimated by measuring three different A1PI concentrations 20 times over a 10 day period. Plasma samples were diluted in assay buffer targeting high, medium and low A1PI concentrations. Assays were limited to 2 per day with at least 3 hours between assays.

Parameter	Coefficient of Variation (%CV)		
	Low	Medium	High
Inter assay	5.8	2.4	1.2
Intra assay	7.0	3.0	1.3
Total	9.0	3.8	1.8

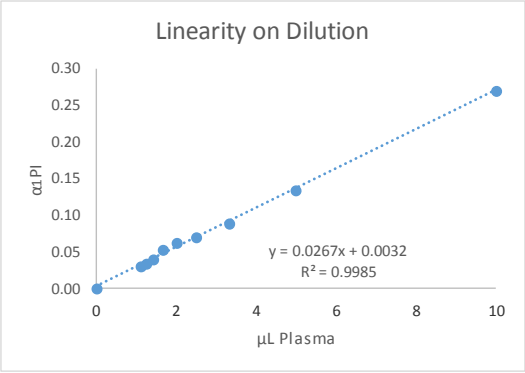
Accuracy:

The recovery of A1PI using this assay was estimated by testing a human plasma sample, an authentic A1PI protein solution and an equal part combination of human plasma with authentic A1PI protein.

Measured A1PI activity in human plasma sample: 0.101 µM
Measured A1PI activity in A1PI protein solution: 0.037 µM
Theoretical (average) A1PI of combined sample: 0.069 µM
Actual measured A1PI activity of combined sample 0.071 µM

Percent recovery of theoretical A1PI = .071/.069 = 103%

Linearity on Dilution:
Dilutions containing human plasma volumes between 10 and 1 µL were tested to assess the linearity of this assay. The graph below shows that the assay is linear at all dilutions within the range of the assay as determined by the relative amount of elastase present in the assay.



a1PI Recovery on Dilution:
2 different plasma samples was diluted 1:50, 1:100, 1:150 and 1:200 and tested for A1PI activity.

Dilution	Plasma 1	Stats	Plasma 2	Recovery
1:50	12.82µM	51%	13.56 µM	67%
1:100	25.42 µM	100%	20.59 µM	100%
1:150	25.64 µM	101%	20.24 µM	99%
1:200	25.02 µM	100%	20.27µM	100%

Note the reduced recovery of A1PI at higher plasma concentrations. We also found the level of reduced recovery of A1PI to be very conserved for specific samples at specific dilutions. We surmise that the cause of this observation may be alpha-2 macroglobulin (A2M), another abundant serum protein with protease activity. Although the exact mechanism has not been determined we believe that at higher concentrations, A2M may be preferentially binding elastase protecting it against A1PI inhibition while preserving its (elastase) ability to react with NSAN reagent thus allowing for increased detection of sample elastase and underestimation of A1PI activity. This recovery data helps underscore the need to properly dilute plasma prior to testing. Other sample types

Sensitivity:
Sensitivity was estimated as 3.29 times the standard deviation for zero A1PI value. *LLD = 37.5 nM Elastase or nM A1PI equivalents. We tested this on a practical level and found 50 nM in reaction mix to be a more reliable estimate of sensitivity for this assay wherein different dilutions showed excellent agreement and recovery.*

Stability:
All components in this assay are stable for 1 year when stored at 4 °C (refrigerated) as specified.

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