K-*ASSAY*®

Alpha-1 AT

For the Quantitative Determination of Human Alpha-1 Anti-Trypsin in Human Serum

Cat. No. KAI-001

INTENDED USE

For the quantitative determination of human alpha-1 anti-trypsin (Alpha-1 AT) in human serum by immunoturbidimetric assay. Alpha-1 anti-trypsin aids in the diagnosis of several conditions including juvenile and adult cirrhosis of the liver. In addition, alpha-1 anti-trypsin deficiency has been associated with pulmonary emphysema. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The **K-*ASSAY*®** Alpha-1 AT assay is intended for the quantitative determination of human alpha-1 anti-trypsin by immunoturbidimetric assay. The antiserum used in the kit was produced against purified human alpha-1 anti-trypsin. The alpha-1 anti-trypsin antibody interacts with the alpha-1 anti-trypsin in the serum forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum alpha-1 anti-trypsin.

Alpha-1 anti-trypsin is also called alpha-1 protease inhibitor because it inhibits trypsin, elastin, and several other proteases. The measurement of alpha-1 anti-trypsin aids in the diagnosis of several conditions including juvenile and adult cirrhosis of the liver.[3] In addition, alpha-1 anti-trypsin deficiency, an autosomal recessive disease, has been associated with pulmonary emphysema.[1,2]

Alpha-1 anti-trypsin has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.[4,5] The **K-*ASSAY*®** Alpha-1 AT assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-*ASSAY*®** Alpha-1 AT assay quantifies the alpha-1 anti-trypsin in the patient's serum based on immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiserum is added to the cuvettes. The samples (antigen) and antiserum are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum alpha-1 anti-trypsin. Following an incubation period lasting approximately 5 min., the absorbance of the solution is measured at 700 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples are interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific for human alpha-1 anti-trypsin.

The **K-*ASSAY*®** Alpha-1 AT assay should be run using a two-reagent clinical chemistry autoanalyzer. Six calibrators are provided in the **K-*ASSAY*®** Multi-Analyte Calibrator. The calibrators are used to prepare a calibration curve for quantifying the levels of alpha-1 anti-trypsin present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

R1: Buffer Reagent 3 x 20 mL
Tris(hydroxymethyl)aminomethane (100 mM)

R2: Antiserum Reagent 2 x 10 mL
Anti-human alpha-1 anti-trypsin goat antiserum (75%)

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Guide Management No. CDC-22 issued by the Center for Disease Control, Atlanta, GA. 1976.

This product contains a chemical known to the State of California to cause cancer. The R-1 reagent contains 0.38% of thiourea (CAS No. 62-56-6).

REAGENT PREPARATION

The reagents are ready to use. They do not need to be reconstituted.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for one month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and / or equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass) within 2 hours. Serum should be stored refrigerated (2-8°C) and can be used within one week or should be stored frozen for up to 2 months.

Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION

Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent 3 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 2 x 10 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of alpha-1 anti-trypsin).

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 700 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

Assay Procedure

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

Sample 3 µL
↓
• ← R1 (Buffer Reagent) 300 µL
↓ 37° 5 min.
• ← R2 (Antiserum Reagent) 100 µL
↓ 37° 5 min.
2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(A1AT)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ( )
R1 VOLUME	(300) ( ) (NO)
R2 VOLUME	(100) ( ) (NO)
WAVELENGTH	( ) (700)
CALIB. METHOD	(NONLINEAR) (4) (6)
STD.(1) Conc.-POS.	(*1) - (1)
STD.(2) Conc.-POS.	(*2) - (2)
STD.(3) Conc.-POS.	(*3) - (3)
STD.(4) Conc.-POS.	(*4) - (4)
STD.(5) Conc.-POS.	(*5) - (5)
STD.(6) Conc.-POS.	(*6) - (6)
SD LIMIT	(999)
DUPLICATE LIMIT	(10000)
SENSITIVITY LIMIT	(0)
ABS. LIMIT (SLOPE)	(32000) (INCREASE)
PROZONE LIMIT	(-32000) (LOWER)
EXPECTED VALUE	(-99999) (99999)
PANIC VALUE	(-99999) (99999)
INSTRUMENT FACTOR	(1.00)

* 1-6 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

For samples with an alpha-1 anti-trypsin level less than 325 mg/dL, a multi-point calibration curve using the **K-*ASSAY*®** Multi-Analyte Calibrator should be used. It is recommended that the user determine calibration frequency as this depends on the instrument and number/type of other assays being performed. Initially, calibration should be performed each day.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls fall outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measurable range for alpha-1 anti-trypsin is between 30 to 325 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the alpha-1 anti-trypsin concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for the dilution.

The absorbance of isotonic saline should be below 0.05 and the absorbance of the 189 mg/dL calibrator should be between 0.20 and 0.42 after allowing for the reagent blank.

PERFORMANCE

Precision

The precision for the ***K-ASSAY*** ® Alpha-1 AT assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

Precision Assay: Within Run

Sample I	Sample II
N = 20	N = 20
Mean = 136	Mean = 343
SD = 3.13	SD = 4.35
CV = 2.3%	CV = 1.27%

Precision Assay: Between Runs

Sample I	Sample II
N = 10	N = 10
Mean = 152	Mean = 355
SD = 2.58	SD = 2.45
CV = 1.70%	CV = 0.69%

Accuracy / Correlation

A comparison of the ***K-ASSAY*** ® Alpha-1 AT and a Binding Site Alpha-1 AT RID Test Kit was performed using a Hitachi 717. The test results provided the following data:

y = 0.708 x + 12.165
r = 0.942
n = 50
x = Binding Site Alpha-1 AT RID
y = ***K-ASSAY*** ® Alpha-1 AT assay

x min = 86	y min = 69
max = 276	max = 213
mean = 208	mean = 160

Assay Range

30-325 mg/dL

INTERFERENCE

Bilirubin F and C:	No interference up to 20 mg/dL
Hemoglobin:	No interference up to 500 mg/dL
Lipemia:	No interference up to 5%

EXPECTED VALUES

The expected value as reported is between 97 to 161 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES

1. Pierce, J.A. "Anti-trypsin and Emphysema," JAMA, 259: 2890-5, 1988.
2. Crystal, RG, "The α-1 Anti-trypsin Gene and its Mutation, Clinical Consequences and Strategies for Therapy," Chest 95: 196-208, 1989.
3. Propst, A. *et al.* "Prognosis and Life Expectancy on α-1 AT Deficiency and Chronic Liver Diseases," Scand. J. Gastroenterol, 30: 1108-1112, Nov. 1995
4. Bergstrom, K., *et al.* "An Automated Turbidimetric Immunoassay for Plasma Proteins." Scand. J. Clin. Lab. Invest., 40:637, 1980.
5. Lizana, J. and Hellina, K., "Manual Immunonephelometric Assay of Proteins with Use of Polymer Enhancement." Clin. Chem. 20:1181, 1974.
6. Killingsworth, L.M. and Savory, J., "Nephelometric Studies on the Precipitin Reactions," J. Clin. Chem., 19: 403-407, 1973.
7. Sternberg, J.C. " A Rate Nephelometer for Measuring Specific Proteins by Immunoprecipitin Reaction," Clin. Chem., 23:1456-64, 1977.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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