K-*ASSAY*®

Alpha-1 AG

For the Quantitative Determination of Human Alpha-1 Acid Glycoprotein in Human Serum

Cat. No. KAI-021

INTENDED USE

For the quantitative determination of human alpha-1 acid glycoprotein (alpha-1 AG) in human serum by immunoturbidimetric assay. Measurement of alpha-1 acid glycoprotein may aid in the diagnosis of collagen (connective tissue) disorders, tuberculosis, infections, extensive malignancy, and diabetes. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

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

Alpha-1 acid glycoprotein, also called orosomucoid, is a 41 kDa glycoprotein. It has a concentration dependant effect on subsets of the immune system. Alpha-1 acid glycoprotein is the best single parameter for the assessment of Crohn's disease (regional enteritis). Increased serum concentrations of alpha-1 acid glycoprotein are associated with inflammation and can be an important diagnostic tool in the diagnosis of rheumatoid arthritis.[1-4]

Alpha-1 acid glycoprotein has been measured using a variety of methods, including radial immunodiffusion and immunoassays. The **K-*ASSAY*®** Alpha-1 AG assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-*ASSAY*®** Alpha-1 AG assay quantifies the alpha-1 acid glycoprotein 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 acid glycoprotein. 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 is interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific for human alpha-1 acid glycoprotein.

The **K-*ASSAY*®** Alpha-1 AG assay should be run using the **K-*ASSAY*®** Multi-Analyte Calibrator. Six calibrators are used to prepare a calibration curve for quantifying the levels of alpha-1 acid glycoprotein present in the patient's serum sample.

KIT COMPOSITION

Reagents (Liquid Stable)

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

R2: Antiserum Reagent 2 x 7 mL
Anti-human alpha-1 acid glycoprotein goat antiserum (70%)

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 Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

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℃). Return all reagents to 2-8℃ 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 1 month if stored at 2-8℃. 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℃) 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 7 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 acid glycoprotein).

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

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) 70 µL
↓ 37 ℃, 5 min.
2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37°C
TEST	(A1AG)
ASSAY CODE	(2 POINT) : (24) - (50)
SAMPLE VOLUME	(3) ( )
R-1 VOLUME	(300) ( ) (NO)
R-2 VOLUME	(70) ( ) (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	(-320000) (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

It is recommended that alpha-1 acid glycoprotein levels be determined using a multi-point calibration curve prepared using the **K-*ASSAY*®** Multi-Analyte Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number 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 acid glycoprotein is between 10 to 200 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 acid glycoprotein 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.

PERFORMANCE

Precision

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

Precision Assay: Within Run

Sample I	Sample II	Sample III
N = 20	N = 20	N = 20
Mean = 32.5	Mean = 92.3	Mean = 233.7
SD = 0.53	SD = 0.6	SD = 2.17
CV = 1.6%	CV = 0.7%	CV = 0.9%

Precision Assay: Between Runs

Sample I	Sample II	Sample III
N = 10	N = 10	N = 10
Mean = 54.6	Mean = 104.7	Mean = 198.6
SD = 0.69	SD = 0.93	SD = 1.37
CV = 1.27%	CV = 0.89%	CV = 0.69%

Accuracy / Correlation

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

y = 1.016x + 3.547
r = 0.995
n = 37
x = Binding Site Alpha-1 AG RID
y = **K-*ASSAY*®** Alpha-1 AG assay

x min	= 35	y min	= 37
max	= 195	max	= 201
mean	= 74	mean	= 79

Assay Range

10 - 200 mg/dL

INTERFERENCE

Ascorbic Acid	No interference up to 200 mg/dL
Bilirubin C	No interference up to 20 mg/dL
Bilirubin F	No interference up to 20 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Intralipid	No interference up to 400 mg/dL

EXPECTED VALUE

The expected value as reported is between 42 to 101 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES

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3. Mercer, D. *et al*. Clin. Chem. 31: 1824-1828, 1985.
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5. Bergstrom, K., *et al*. "An Automated Turbidimetric Immunoassay for Plasma Proteins." Scand. J. Clin. Lab. Invest., 40:637, 1980.
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7. Killingsworth, L.M. and Savory, J., "Nephelometric Studies on the Precipitin Reactions," J. Clin. Chem., 19: 403-407, 1973.
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LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or “Use By” Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostics Use
2-8 °C	Temperature Limitation. Store between 2 and 8 degrees C
	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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