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 immununoturbidimetric 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 (alpha-1 AG). The alpha-1 acid glycoprotein is an acute-phase protein that can be used to diagnose collagen (connective tissue) disorders. It is present in serum at a concentration of approximately 100 mg/L and is not found in other body fluids. It is responsible for the transport and clearance of several low-molecular-weight proteins, including fibrinogen. Two forms of the protein are present: the native glycoprotein and the prozone. The native form has a molecular weight of 110 kDa, while the prozone form has a molecular weight of 55 kDa.

The K-ASSAY® Multi-Analyte Calibrator is a standardized human serum with a known concentration of alpha-1 acid glycoprotein. It is used to calibrate the instrument and to monitor the accuracy of the assay. The calibrator contains a mixture of proteins, including alpha-1 acid glycoprotein, and is used to determine the linearity of the instrument and the precision of the assay.

The assay is performed using a two-reagent automated analyzer that measures the absorbance of the solution at 700 nm. The absorbance is measured in the presence of antiserum and is used to determine the concentration of alpha-1 acid glycoprotein in the sample.

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:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and / or equipment to be used
- Operational precautions, limitations, and hazards

SERVICE AND MAINTENANCE INFORMATION

SPECIMEN COLLECTION AND PREPARATION
Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum should 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
Reagent 2 (R-2) Antiserum Reagent

Materials Required But Not Supplied
Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KAI-016G (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°C

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

An example of an automated application (Hitachi 717):
- Sample: 3 µL
- R1 (Buffer Reagent): 300 µL, 37°C, 5 min.
- R2 (Antiserum Reagent): 70 µL, 37°C, 5 min.

Use 2-point endpoint, 700 nm

2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT
Hitachi 717
TEMPERATURE
37°C
TEST
(A1AG)
ASSAY CODE
(2 POINT) : (24) - (50)
SAMPLE VOLUME
3 ( )
R1 VOLUME
300 ( ) (NO)
R2 VOLUME
70 ( ) (NO)
WAVELENGTH
32000 ( NO)
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
(52000) (LOWER)
EXPECTED VALUE
(-99999) (99999)
PANIC VALUE
(-99999) (99999)
INSTRUMENT FACTOR
(1.00)

NOTE
* 1-6 Inlet concentration of calibrators
Parameters for other automated analyzers are available.

CALIBRATION
A multi-point calibration curve using the K-ASSAY® Multi-Analyte Calibrator is recommended. 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.

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The K-ASSAY® Alpha-1 AG assay is intended for the quantitative determination of human alpha-1 acid glycoprotein (alpha-1 AG). The assay is automated and uses a two-reagent format.

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DUPLICATE LIMIT
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SENSITIVITY LIMIT
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ABS LIMIT (SLOPE)
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**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. The absorbance of isotonic saline should be below 0.2 and the absorbance of the 80 mg/dL calibrator should be between 0.05 and 0.30 after allowing for the reagent blank.

**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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 20</td>
<td>N = 20</td>
<td>N = 20</td>
</tr>
<tr>
<td>Mean = 32.5</td>
<td>Mean = 92.3</td>
<td>Mean = 233.7</td>
</tr>
<tr>
<td>SD = 0.53</td>
<td>SD = 0.6</td>
<td>SD = 2.17</td>
</tr>
<tr>
<td>CV = 1.6%</td>
<td>CV = 0.7%</td>
<td>CV = 9.0%</td>
</tr>
</tbody>
</table>

**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:

\[
y = 1.016x + 3.547 \\
r = 0.995 \\
n = 37 \\
x = Binding Site Alpha-1 AG RID \\
y = K-ASSAY® Alpha-1 AG assay \\
x min = 35 \\
y min = 37 \\
max = 195 \\
mean = 74 \\
max = 201 \\
mean = 79
\]

**ASSAY RANGE**

10-200 mg/dL

**INTERFERENCE**

- Ascorbic Acid: No interference up to 200 mg/dL
- Bilirubin F and C: No interference up to 20 mg/dL
- Hemoglobin: No interference up to 500 mg/dL
- Lipemia: No interference up to 4%

**EXPECTED VALUES**

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

**REFERENCES**


**LABELING SYMBOLS**

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

**EU AUTHORIZED REPRESENTATIVE**

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