**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 antisera 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. In addition, alpha-1 anti-trypsin deficiency, an autosomal recessive disease, has been associated with pulmonary emphysema.  

Alpha-1 anti-trypsin has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometry, and enzyme-linked immunosorbent assay. 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. Micromolecules of serum and antigen diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiseraum is added to the cuvettes. The samples (antigen and antiseraum) 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 antiseraum 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® Alpha-1 AT kit. 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  
- R2: Antiserum Reagent 2 x 10 mL  

**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.  

**WARNING:** This product can expose you to chemicals including thiourea which is known to the State of California to cause cancer/birth defects or other reproductive harm. For more information go to www.P65Warnings.ca.gov. The R-1 reagent contains 0.009% 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:

- 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**  
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 sample 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**
- 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):

<table>
<thead>
<tr>
<th>Sample</th>
<th>3 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1 (Buffer Reagent)</td>
<td>300 µL</td>
</tr>
<tr>
<td>R2 (Antiserum Reagent)</td>
<td>100 µL</td>
</tr>
</tbody>
</table>

2-point endpoint, 700 nm

**Automated Method (Example)**

**Chemistry Parameters for Automatic Analyzer**

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Roche / Hitachi 717</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST</td>
<td>(A1AT)</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT) : (24) - (50)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(3)</td>
</tr>
<tr>
<td>R-1 VOLUME</td>
<td>(300) (NO)</td>
</tr>
<tr>
<td>R-2 VOLUME</td>
<td>(100) (NO)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(700)</td>
</tr>
<tr>
<td>CALIB. METHOD</td>
<td>(NONLINEAR) (4) (6)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(5)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(6)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>ABS. LIMIT (SLOPE)</td>
<td>(32000) (INCREASE)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(320000) (LOWER)</td>
</tr>
<tr>
<td>EXPECTED VALUE</td>
<td>(-99999) (-99999)</td>
</tr>
<tr>
<td>PANC VALUE</td>
<td>(-99999) (-99999)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>(1.0)</td>
</tr>
</tbody>
</table>

*1-6: Input concentration of calibrators.
Parameters for other automated analyzers are available.
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:

\[
\begin{align*}
    y &= 0.708 x + 12.165 \\
    r &= 0.942 \\
    n &= 50 \\
    x &= \text{Binding Site Alpha-1 AT RID} \\
    y &= \text{K-ASSAY® Alpha-1 AT assay} \\
\end{align*}
\]

\[
\begin{align*}
    x_{\text{min}} &= 86 & y_{\text{min}} &= 69 \\
    x_{\text{max}} &= 276 & y_{\text{max}} &= 213 \\
    \text{mean} &= 208 & \text{mean} &= 160
\end{align*}
\]

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

**REFERENCES**