**K-ASSAY®**

**Insulin**

*For the Quantitative Determination of Human Insulin in Serum and Plasma*

[Cat. No. KAI-040 and KAI-071]

**INTENDED USE**

For the quantitative determination of human insulin in serum and plasma by immunoturbidimetric assay. **IN VITRO** DIAGNOSTIC USE.

**SUMMARY**

Insulin is a peptide hormone with approximate molecular weight of 5,800 daltons. Secreted from β cells of the islet of Langerhans in the pancreas, insulin acts to reduce the blood sugar level. Since the blood insulin level reflects the function of β cells, insulin has been widely used as an important diagnostic tool for diabetes mellitus. The K-ASSAY® Insulin test is a highly specific assay for insulin in serum or plasma.

**PRINCIPLE OF TEST**

Latex particles coated with antibody specific to human insulin form immune complexes in the presence of insulin from the sample. The immune complexes cause an increase in light scattering that is proportional to the concentration of insulin in the serum or plasma sample. The light scattering is measured by reading turbidity at 650 nm primary, 800 nm secondary. The sample insulin concentration is determined versus insulin calibrators of known concentrations.

**KIT COMPOSITION**

Reagents (Liquid Stable)
- **R1**: Buffer Reagent
- **R2**: Latex Suspension

Anti-human insulin mouse monoclonal antibody, ~1 mg/mL

**WARNINGS AND PRECAUTIONS**

FOR IN VITRO DIAGNOSTIC USE. Re 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 (≤0.1 wt%) as 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.
- Invert R1 and R2 bottles several times gently prior to use.
- Remove the foam prior to measurement, if necessary.

**REAGENT PREPARATION**

Reagents are ready to use and do not require reconstitution.

**STORAGE AND HANDLING**

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. The reagents can be used until the expiration date. The shelf life of this product is one year recommended that specimen collection be carried out in accordance with NCCLS document M29-A2. Avoid repeated freeze/thaw cycles. Serum or plasma may be stored refrigerated (2-8°C) for up to a week. For long-term storage, keep at -20°C or below.

**AUTOMATED ANALYZER APPLICATION**

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

**PROCEDURE**

**Materials Supplied**

KAI-040 Insulin
- Reagent 1 (R-1) Buffer Reagent 1 x 13.5mL
- Reagent 2 (R-2) Latex Suspension 1 x 5mL

KAI-071 Insulin (L)
- Reagent 1 (R-1) Buffer Reagent 2 x 13.5mL
- Reagent 2 (R-2) Latex Suspension 2 x 5mL

**Materials Required But Not Supplied**

Calibrator: K-ASSAY® Insulin Calibrator, Cat. No. KAI-072C

- Purified water
- Clinical chemistry analyzer capable of accurately reading at 600 nm (main) and 800 nm (sub), accurately dispensing the required volumes, and maintaining 37°C.

**Assay Procedure**

An example of automated application:

- **Sample** 12 µL
  - R1 (Buffer Reagent) 135 µL
  - R2 (Latex Reagent) 50 µL
- Measure 2 Point End at 600 nm main, 800 nm sub (if available)

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

**SPECIMEN COLLECTION AND PREPARATION**

**Serum**

Blood should be collected from a patient and the serum separated as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the separated serum from the clot to a plastic tube (not glass) within 2 hours. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-A2.

Avoid repeated freeze/thaw cycles.

**Plasma**

Whole blood is collected in sodium citrate, sodium EDTA and sodium fluoride anticoagulant. After collection, immediately centrifuge. In the U.S., follow NCCLS guideline H3-A2. Avoid repeated freeze/thaw cycles.

Serum or plasma may be stored refrigerated (2-8°C) for up to a week. For long-term storage, keep at -20°C or below.

**CALIBRATION**

It is recommended that a multi-point calibration curve be made using the K-ASSAY® Insulin Calibrator (KAI-072C). 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 with every assay performed. The value determined for the controls should fall within the stated limits of the values assigned to the controls. The validity of the assay is in question if the values for the controls generated by the assay’s calibration curve do not fall within the range. Recalibrate if the values determined for the controls fall outside the stated range.

**LIMITATIONS OF PROCEDURE**

Use DI water for standard 1. Use Insulin calibrators 1-5 for standards 2-6. *2-6: Input concentration of calibrators (using one decimal place [X.X]).

# = User Defined

Parameters for other automated analyzers are available.
**PERFORMANCE**

**Sensitivity**
When a saline blank is used as a sample, the absorbance change is ≤ 0.0023/min. When a calibrator, having an insulin concentration of around 20 µIU/mL, is assayed, the absorbance (after subtracting the saline blank) is within the range of 0.003 to 0.038/min.

**Specificity**
When a sample with a known value is assayed, the result is within ±15% of the assigned value.

**Precision**
When a sample is assayed 5 times (within-run), the absorbance C.V. is ≤ 10%.

(Within Run)
The following results were obtained on a Hitachi 917 analyzer with human serum:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample II</th>
<th>Sample III</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean (µIU/mL)</td>
<td>18.81</td>
<td>27.82</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.224</td>
<td>0.192</td>
</tr>
<tr>
<td>CV</td>
<td>1.19%</td>
<td>0.69%</td>
</tr>
</tbody>
</table>

(Between Runs)
The following results were obtained on a Hitachi 917 analyzer with human serum:

<table>
<thead>
<tr>
<th>Sample IV</th>
<th>Sample V</th>
<th>Sample VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Mean (µIU/mL)</td>
<td>12.21</td>
<td>25.90</td>
</tr>
<tr>
<td>Std. Dev.</td>
<td>0.530</td>
<td>0.777</td>
</tr>
<tr>
<td>CV</td>
<td>4.34%</td>
<td>3.00%</td>
</tr>
</tbody>
</table>

**Accuracy / Correlation**
A comparison of the K-ASSAY® Insulin and another company’s Insulin EIA was performed with the following results:

Serum Sample
\[ y = 0.8061x + 2.8674 \]
\[ r = 0.997 \]
\[ n = 32 \]
\[ x = \text{another company’s insulin assay} \]
\[ y = \text{K-ASSAY® Insulin Assay} \]

Plasma Sample
\[ y = 0.8161x + 4.5552 \]
\[ r = 0.986 \]
\[ n = 47 \]
\[ x = \text{another company’s insulin assay} \]
\[ y = \text{K-ASSAY® Insulin Assay} \]

**Assay Range**
1.0 to 100 µIU/mL (or value of highest calibration point)

**Lower Limit of Detection**
The analytical sensitivity is 1 µIU/mL. This means that when saline and serum containing 1 µIU/mL of insulin are tested 10 times, ± 2.6 SD of the respective results do not overlap each other.

**INTERFERENCE**
No cross-reactivity with pro-insulin was observed. Hemoglobin, bile or rheumatoid factor did not interfere with the assay.

<table>
<thead>
<tr>
<th>Component</th>
<th>Interference Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin F</td>
<td>No interference up to 19.3 mg/dL</td>
</tr>
<tr>
<td>Bilirubin C</td>
<td>No interference up to 19.9 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>No interference up to 450 mg/dL</td>
</tr>
<tr>
<td>Lipemia</td>
<td>No interference up to a formazin turbidity of 1,550</td>
</tr>
<tr>
<td>RF</td>
<td>No interference up to 450 IU/mL</td>
</tr>
</tbody>
</table>

**PROZONE**
No hook effect seen up to at least 1,000 µIU/mL.

**EXPECTED VALUES**
The expected range for fasting insulin concentration has been reported to be up to 20 to 35 µIU/mL (RIA).

**REFERENCES**

**LABELING SYMBOLS**
- Lot Number
- Reagent
- Expiration or “Use By” Date
- Catalog Number
- 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
- Authorized Representative in the European Community

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