The Insulin Quantitative Test Kit is based on a solid phase enzyme-immunoassay (EIA) principle. The test is used to determine the concentration of Insulin in human serum.

**Materials and Components**

- **Materials provided with the test kit:**
  - Antibody coated microtiter plate 96 wells
  - Enzyme Conjugate Reagent 12 mL
  - TMB Substrate 12 mL
  - Stop Solution 12 mL
  - Wash Concentrate (50X) 15 mL
  - Insulin reference standards, containing 0, 5, 25, 50, 100, and 200 µIU/mL in liquid form (ready to use) or lyophilized form (the standard calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 1st IRR 66/304)

- **Materials required but not provided:**
  - Precision pipettes, 40-200 µL, 200-1000 µL
  - Disposable pipette tips
  - Distilled water
  - Vortex mixer
  - Absorbent paper or paper towel
  - Microtiter plate reader
  - Graph paper
  - Microplate Reader with 450nm and 620nm wavelength absorbance capability (The 620nm filter is optional)
  - Dispenser(s) for repetitive deliveries of 0.100 mL and 0.300 mL volumes with a precision of better than 1.5% (optional)

**Calculation of Results**

Calculate the mean absorbance value for each set of Insulin reference standards, specimens, and controls. The absorbance values are used to construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in units per mL on linear graph paper. The standard curve and the absorbance values for each specimen are used to determine the corresponding concentration of Insulin in units per mL from the standard curve. Any diluted specimens must be corrected for by the appropriate dilution factor.
EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against Insulin concentrations shown in the X axis.

<table>
<thead>
<tr>
<th>Insulin Values (µU/mL)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.075</td>
</tr>
<tr>
<td>5</td>
<td>0.148</td>
</tr>
<tr>
<td>25</td>
<td>0.497</td>
</tr>
<tr>
<td>50</td>
<td>0.955</td>
</tr>
<tr>
<td>100</td>
<td>1.716</td>
</tr>
<tr>
<td>200</td>
<td>3.039</td>
</tr>
</tbody>
</table>

LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
4. Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction.
5. Plate readers measure vertically. Do not touch the bottom of the wells.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Highly lipemic, hemolysed or grossly contaminated specimen(s) should not be used.
8. Patient samples with Insulin concentrations above 200 µU/mL may be diluted with the zero calibrator and re-assayed. Multiply the value obtained by the dilution factor to obtain the corrected value.
9. Use components from the same lot. No intermixing of reagents from different batches.

EXPECTED VALUES

Insulin values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese nondiabetic individuals, insulin levels are higher in obese non-diabetic subjects and lower in trained athletes.

Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method. Based on the clinical data gathered by Monobind in concordance with the published literature the following ranges have been assigned.

These ranges should be used as guidelines only:
- Children < 12 yrs: < 10 µU/mL
- Adult (Normal): 0.7-9.0 µU/mL
- Diabetic (Type II): 0.7-25 µU/mL

SENSITIVITY

The sensitivity (detection limit) was ascertained by determining the variability of the 0 µU/mL serum calibrator and using the 2SD (95% certainty) statistic to calculate the minimum dose. The assay sensitivity was found to be 2.0 µU/mL.

REFERENCES