The antiserum is developed in guinea pig using bovine insulin as the immunogen. The product is provided as a undiluted antiserum that contains 0.1% sodium azide (see MSDS)* as a preservative.

**Working Dilution:** 1:80,000
Dilute the antiserum to the working dilution in 0.05 M potassium phosphate buffered saline, pH 7.4, containing 0.5% human serum albumin and 0.1% sodium azide.

**Immunohistology**
A dilution of 1:100 was determined by indirect immunohistology using formalin-fixed, paraffin-embedded human pancreas, biotin conjugated anti-guinea pig IgG and ExtrAvidin-Peroxidase.

**Storage**
Store undiluted antiserum at −20°C, in working aliquots. Repeated freezing and thawing is not recommended. During periods of continuous, store at 2-8°C.

**RIA SYSTEM**

**RIA Characterization**
The antiserum is characterized utilizing the following dextran coated charcoal radioimmunoassay (RIA) protocol, where 0.5 ml of diluted antiserum has been found to bind at least 40% of 100 picograms of iodinated (¹²⁵I) pig insulin with a specific activity of approximately 100µCi/µg.

It is recommended that the antiserum first be evaluated in the assay system described due to differences in systems and procedures.

**RIA Reagents**

**(A)** Standards: Prepare a stock standard solution of 0.1 mg/ml pig insulin (Sigma Product No. I-3505) in 0.7% acetic acid. Dilute a portion of the stock solution with buffer (B) to a concentration of 20 mIU/ml according to the stated potency of the product (approximately 24 IU/mg). Freeze in working aliquots to avoid repeated freezing and thawing. Dilute an aliquot of the 20 mIU/ml standard with insulin-free plasma (D) to the following concentrations: 20, 10, 5, 2.5, 1.25, 0.625 and 0.312 µIU/0.1ml.

**Note:** The antiserum may be used for the determination of insulins of various origins due to the high cross-reactivity of the antiserum to such insulins. If high accuracy is required, it is recommended that a standard homologous to the insulin under study be used.

**(B)** Dilution buffer: potassium phosphate buffered saline, pH 7.4, containing 0.5% human serum albumin (Sigma Product No. A-1887) and 0.1% sodium azide. 

**Note:** Before the addition of human serum albumin, set aside a portion of the buffer for use in the preparation of the dextran coated charcoal suspension (C).

**(C)** Dextran coated charcoal suspension: 0.5% activated charcoal untreated powder 250-350 mesh (Sigma Product No. C5260), 0.1% dextran approximate average molecular weight 70,000 (Sigma Product No. D1390) in buffer (B) without human serum albumin. It is important that the dextran be in solution before the addition of charcoal. The dextran coated charcoal suspension should be stirred and kept at 0°C in ice-water for at least 30 minutes before and during use.

**(D)** Insulin-free plasma¹: To 10 ml of human plasma add 1g charcoal and stir gently for 1 hour at 0°C. Centrifuge at 1400 x g for 30 minutes at 4°C. If charcoal particles remain in suspension, the insulin-free plasma may be filtered. Store insulin-free plasma at −20°C, in working aliquots.

**RIA Protocol**
In polypropylene test tubes add 0.1 ml sample or standard (A) and 0.5 ml diluted antiserum.

Vortex the tubes.

Incubate for 5 hours at 2-8°C.

Add 0.1 ml radioactive tracer diluted in dilution buffer (B).

Vortex the tubes.

Incubate for 18-20 hours at 4°C.

Rapidly add 0.5 ml cold dextran coated charcoal suspension (C) to each tube.

Vortex the tubes.

Incubate for 10 minutes at 0°C in ice-water.

Centrifuge at 2000 x g for 15 minutes at 4°C.

Remove supernatant from each tube, add scintillation cocktail to the supernatant and determine the amount of radioactivity present.

**RIA Specificity**

Specificity of the antiserum is defined as the ratio of antigen concentration to cross-reactant concentration at 50% inhibition of maximum binding. The cross-reactivity data obtained in the described RIA system is as follows:

<table>
<thead>
<tr>
<th>Cross-Reactant</th>
<th>%Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Chorionic Gonadotropin</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glucagon</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Bovine Insulin</td>
<td>100</td>
</tr>
<tr>
<td>Human Insulin</td>
<td>100</td>
</tr>
<tr>
<td>Human Luteinizing Hormone</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**RIA Sensitivity**

Sensitivity is defined as the 90% intercept of a B/B₀ standard curve. In the above system the sensitivity has been found to be µU bovine insulin per tube.

**RIA Affinity Constant**

The affinity constant (Kₐ) is determined by a Scatchard plot using the described RIA system.

\[
K_a = 2.0 \times 10^{10} \text{ L/mole.}
\]

**Insulin Levels**

<table>
<thead>
<tr>
<th>µIU/0.1ml</th>
<th>After overnight fasting (healthy subjects)²</th>
<th>Peak in intravenous glucose tolerance testing²</th>
<th>After ingestion of 100g glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1-2.5</td>
<td>6.8-8.6</td>
<td>4.0-25.0</td>
</tr>
</tbody>
</table>

**References**


*Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

3/98