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ProductInformation

ANTI-PIG INSULIN
Developed in Guinea Pig
Whole Antiserum

Product No. **I 8510**

The antiserum is developed in guinea pig using purified pig insulin as the immunogen. The product is provided as a whole antiserum with 0.1% sodium azide (see MSDS)* as preservative.

Working Dilution: Minimum 1:80,000
Dilute the antiserum to the working dilution in 0.05 M PBS, pH 7.4, containing 0.5% human serum albumin (HSA) and 0.1% sodium azide.

Storage
For continuous use, store at 2-8 °C for a maximum of one month. For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

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 (^{124}I) pig insulin with a specific activity of approximately 100 µCi/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 1mg pig insulin/ml of 0.7% acetic acid. Dilute to 20 mU/ml in buffer (B) according to the stated potency of the material (usually 25 U/mg). Before each assay thaw one aliquot and dilute 1:100 with insulin free plasma to obtain the 200 µU/ml standard. Prepare serial dilutions using the insulin-free plasma from the 200µU standard to give the followiing standard dilutions: 100, 50, 25, 12.5, 6.25 and 3.12 µU/ml.

(B) Dilution buffer: 0.05 M PBS, pH 7.4, containing 0.5% human serum albumin (HSA) and 0.1% sodium azide.
Note: Before addition of HSA put aside some buffer which will be used in the preparation of the dextran coated charcoal suspension.

(C) Dextran coated charcoal suspension: 2.5% Activated Charcoal Untreated Powder 100-400 mesh (Sigma Product No. C 5260), 0.25% dextran approximate average molecular weight 70,000 (Sigma Product No. D 1390) in buffer (B). 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 1 hour before and during use.

(D) Insulin-Free Plasma[1]: To 10 ml of human plasma add 1 gram of charcoal, shake or stir gently at 0 °C for 1 hour. Centrifuge at 2500 rpm, 4 °C for 30 minutes, to seperate the plasma. If particles or charcoal remain in the plasma, either ultracentrifuge at 40,000 rpm for 10 minutes, or filter the plasma. Store at –20 °C in 2 ml aliquots.

(E) Radiolabeled Tracer: Dilute the ^{125}I-Insulin (approximately 100 µCi/µg) with buffer (B) to a concentration of 1 ng/ml.

RIA Protocol
1. In polypropylene test tubes add 0.1 ml sample or standard (A) and 0.5 ml diluted antiserum.
2. Prepare a total tube, a zero control and a standard blank. To these tubes add 0.1 ml of insulin-free plasma and 0.5 ml buffer.
3. Vortex the tubes.
4. Incubate at 4 °C for 5 hours.
5. Add 0.1 ml tritiated radioactive tracer diluted in dilution buffer (B).
6. Vortex the tubes.
7. Incubate for overnight (18-20 hours) at 4 °C.
8. Rapidly add 0.5 ml cold dextran coated charcoal suspension (C) to each tube.
9. Vortex the tubes.

10. Incubate for 10 minutes at 0 °C in ice-water.
11. Centrifuge at 3000 x g for 15 minutes at 4 °C.
12. Remove 1.0 ml supernatant from each tube, add scintillation cocktail to the supernatant and determine the amount of radioactivity present.

Calculations

$$\%B/B_0 = \frac{\text{cpm sample} - \text{cpm sample blank}}{\text{cpm zero tube} - \text{cpm standard blank}} \times 100$$

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:

Cross-Reactant	**%Cross-Reactivity**
Pig Insulin	100
Bovine Insulin	100
Human Insulin	100
Glucagon	<0.01

RIA Sensitivity
Sensitivity is defined as the 90% intercept of a B/B_0 standard curve. In the above system the sensitivity has been found to be 5 µU pig insulin/tube.

RIA Affinity Constant
The affinity constant (K_a) is determined by a Scatchard plot using the described RIA system.
$K_a = 1.5 \times 10^{10}$ L/mole.

Immunohistology
A working dilution of 1:100 was determined by indirect immunoperoxidase staining of human pancreas.

Insulin Levels	µU/ml
After overnight fast (healthy subjects)[2]	1-25
Peak in IV glucose tolerance tests[2]	68-86
After ingestion of 100 g of glucose[3]	40-250

References

1. Albano, J.D.M., et al., Acta Endocrinologica, **70**, 487 (1972).
2. Starr, J.I. and A.H. Rubenstein in "Methods of Hormone Radioimmunoassay", Yaffee and Bermand eds., Academic Press Inc., New York, pp. 289-311 (1974).
3. Yalow, R.S. and S.A. Berson in "Methods in Radioimmunoassay of Peptide Hormones", Yalow, ed., North Holland Publishing Co., Amsterdam-Oxford, pp. 168-174 (1976).

*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.

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