Product information

User´s Manual

Insulin (Rat) ELISA

For detection of Insulin in rat serum, plasma, body fluids,
tissue lysates or cell culture supernates.

Catalog No.:	**BE69090**
▽Σ	**96**
Storage:	**2-8°C**

RUO **For Research Use Only – Not for Use in Diagnostic Procedures.**

Manufactured for:
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1 INTRODUCTION

1.1 Intended Use

For quantitative detection of Insulin in Rat serum, plasma, body fluids, tissue lysates or cell culture supernates. For research use only, not for use in diagnostic procedures.

1.2 Background

Insulin, synthesized by the beta cells of the islets of Langerhans, consists of 2 dissimilar polypeptide chains, A and B, which are linked by 2 disulfide bonds. The human insulin gene contains 3 exons; exon 2 encodes the signal peptide, the B chain, and part of the C peptide, while exon 3 encodes the remainder of the C peptide and the A chain. Insulin has a potent acute antiinflammatory effect, including a reduction in intranuclear NF-kappa-B, an increase in IKB, and decreases in the generation of reactive oxygen species. It causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen inside these tissues, and improved insulin-stimulated glucose uptake after endurance training results from hemodynamic adaptations as well as increased cellular protein content of individual insulin signaling components and molecules involved in glucose transport and metabolism.

2 PRINCIPLE OF THE TEST

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Anti-Insulin monoclonal antibody is pre-coated onto 96-well plates. HRP conjugated anti-Insulin polyclonal antibody are used as detection antibodies. The standards, test samples and HRP conjugated detection antibody are added to the wells subsequently, and wash with wash buffer. TMB substrates are used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the Insulin amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of Insulin can be calculated.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only, not for use in diagnostic procedures.
2. Before the experiment, centrifuge each kit component for several minutes to bring down all reagents to the bottom of tubes.
3. It is recommended to measure each standard and sample in duplicate.
4. Do NOT let the plate completely dry at any time! Since the dry condition can inactivate the biological material on the plate.
5. Do not reuse pipette tips and tubes to avoid cross contamination.
6. Do not use the expired components and the components from different batches.
7. The TMB substrate A & B (Kit Component 4 & 5) is colorless and transparent before use, if not, please contact us for replacement.

4 REAGENTS

4.1 Reagents provided

1. One 96-well plate pre-coated with anti-Rat Insulin antibody
2. Insulin standards: 6 tubes, 0.5ml/tube (0 uIU/ml, 8 uIU/ml, 16 uIU/ml, 32 uIU/ml, 80 uIU/ml, 140 uIU/ml)
3. HRP conjugated anti-Rat Insulin antibody (RTU): 6 ml
4. TMB substrate A: 7 ml
5. TMB substrate B: 7 ml
6. Stop solution: 7 ml
7. Wash buffer (25X): 30ml

4.2 Materials required but not provided

1. 37℃ incubator
2. Microplate reader (wavelength: 450nm)
3. Precise pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml of Eppendorf tubes
7. Plate cover
8. Absorbent filter papers
9. Plastic or glass container with volume of above 1L

4.3 Storage Conditions / Expiration

Store at 2-8℃ for 12 months

4.4 Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20℃ for long term. Avoid multiple freeze-thaw cycles.

✧ **Body fluids, tissue lysates and cell culture supernatants:** Centrifuge to remove precipitate, analyze immediately or aliquot and store at -20℃.

✧ **Serum:** Coagulate the serum at room temperature (about 2 hours) or at 4°C overnight. Centrifuge at approximately 2000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20℃.

✧ **Plasma:** Collect plasma with EDTA as the anticoagulant. Centrifuge for 30 min at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20° C.

Note: 1. Coagulate blood samples completely, then, centrifuge, and avoid hemolysis and particle.
2. NaN_3 cannot be used as test sample preservative, since it is the inhibitor for HRP.
3. After collecting samples, analyze immediately or aliquot and store frozen at -20°C. Avoid repeated freeze-thaw cycles.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1:25) with distilled water (i.e. add 30ml of concentrated wash buffer into 720ml of distilled water).

5 ASSAY PROCEDURE

5.1 Test Procedure

Mix thoroughly when diluting the reagents. It is recommended to plot a standard curve for each test. End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate.
2. Aliquot 50 µl of 0uIU/mL, 8 uIU/ml, 16 uIU/ml, 32 uIU/ml, 80 uIU/ml, 140 uIU/ml standard solutions into the standard wells. (Note: Add user's 50µl of sample diluent buffer buffer into the control well).
3. Add 50 µl of properly diluted sample (Rat serum, plasma, body fluids, tissue lysates or cell culture supernatants) into test sample wells.
4. Add 50 µl of HRP conjugated anti-Rat Insulin antibody into the above wells (Except the control well). Add the solution at the bottom of each well without touching the side wall.
5. Seal the plate with a cover and incubate at 37℃ for 60 min.
6. Remove the cover, discard the liquid of wells, clap the plate on the absorbent filter papers or other absorbent material.
7. Wash plate 3 times with Wash buffer (Kit Component 7) using one of the following methods:

   Manual Washing: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with Wash buffer (Kit Component 7) buffer and vortex mildly on ELISA shaker for 2 min, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure two more times for a **total of THREE washes**.

   Automated Washing: Aspirate all wells, then wash plate **THREE times** with Wash buffer (Kit Component 7) (overfilling wells with the buffer). After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min or shaking.
8. Add 50 µl of TMB substrate A (Kit Component 4) into each well, and then, add 50 µl of TMB substrate B (Kit Component 5), vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds), cover the plate and incubate in dark at 37°C for 15 min. (Note: This incubation time is for reference use only, the optimal time should be determined by end user.) And the shades of blue can be seen in the first 3-4 wells (with most concentrated Rat Insulin standard solutions), the other wells show no obvious color.
9. Add 50 µl of Stop solution (Kit Component 6) into each well and mix thoroughly. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min after adding the stop solution.

5.2 Results

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat Insulin concentration of the samples can be interpolated from the standard curve.

Note:

1. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.
2. If end user hopes to calculate with unite * ng/ml, please multiple the $O.D._{450}$ values by 0.04167.

6 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. It is recommended to use controls according to state and federal regulations. The use of controls is advised to assure the day to day validity of results. It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results. Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials, results of unknowns should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods. After checking the above-mentioned items without finding any error contact your distributor or IBL-America directly.

7 PERFORMANCE CHARACTERISTICS

7.1 Range

0 uIU/ml - 140 uIU/ml

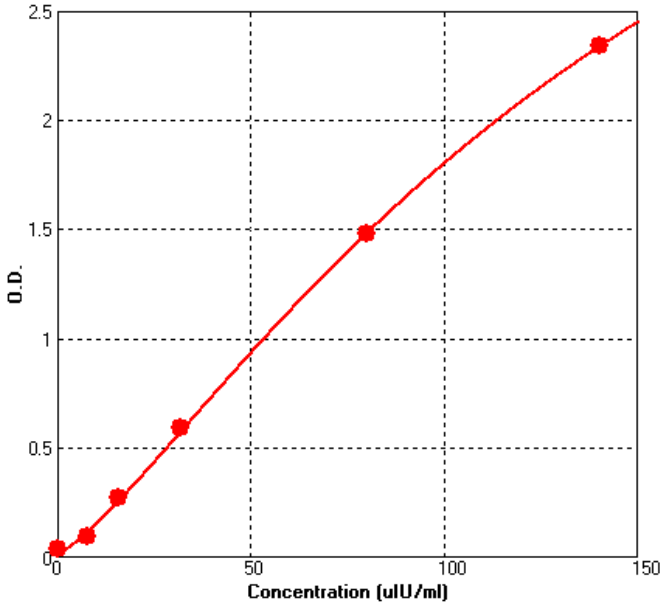
7.2 Sensitivity

< 5 uIU/ml

7.3 Typical Data & Standard Curve

Results of a typical standard run of a Rat Insulin ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment. (N/A=not applicable)

X	uIU/ml	0	8	16	32	80	140
Y	OD450	0.037	0.095	0.275	0.593	1.483	2.342



7.3 References:

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2. Dandona, P., Aljada, A., Mohanty, P., Ghanim, H., Hamouda, W., Assian, E., Ahmad, S. Insulin inhibits intranuclear nuclear factor kappa-B and stimulates I-kappa-B in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? J. Clin. Endocr. Metab. 86: 3257-3265, 2001.

3. Frosig, C., Rose, A. J., Treebak, J. T., Kiens, B., Richter, E. A., Wojtaszewski, J. F. P. Effects of endurance exercise training on insulin signaling in human skeletal muscle: interactions at the level of phosphatidylinositol 3-kinase, Akt, and AS160. Diabetes 56: 2093-2102, 2007.

8 ORDERING INFORMATION

This kit is manufactured for Immuno-Biological Laboratories, Inc. (IBL-America). For ordering information, please contact:

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