
Product Manual

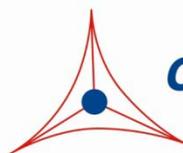
Human Insulin ELISA Kit

Catalog Numbers

MET- 5063 96 assays

MET- 5063- 5 5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Insulin is a peptide hormone that regulates metabolism of carbohydrates, fats, and proteins through the absorption of glucose from the blood into muscle, fat, and liver cells. Produced by beta cells in the pancreatic islets, insulin affects protein synthesis in many tissues. This anabolic behavior results in glucose being converted to glycogen or fats via glycogenesis and lipogenesis respectively. High blood glucose levels result in beta cell secretion of insulin, whereas alpha cells counteract low blood glucose levels by secreting glucagon. The hypoglycemic effects of insulin are balanced by hyperglycemic hormones such as glucagon, cortisol, epinephrine, and growth hormone. Insulin's actions influence many pathways such as increased DNA replication, increased lipid and protein synthesis, increased glycogen synthesis, increased potassium uptake, decreased gluconeogenesis and renal sodium excretion, decreased proteolysis, autophagy, lipolysis. Insulin accentuates memory function and cognition once in the brain. Disruption of this balance of blood glucose results in several pathological disease states, such as diabetes mellitus.

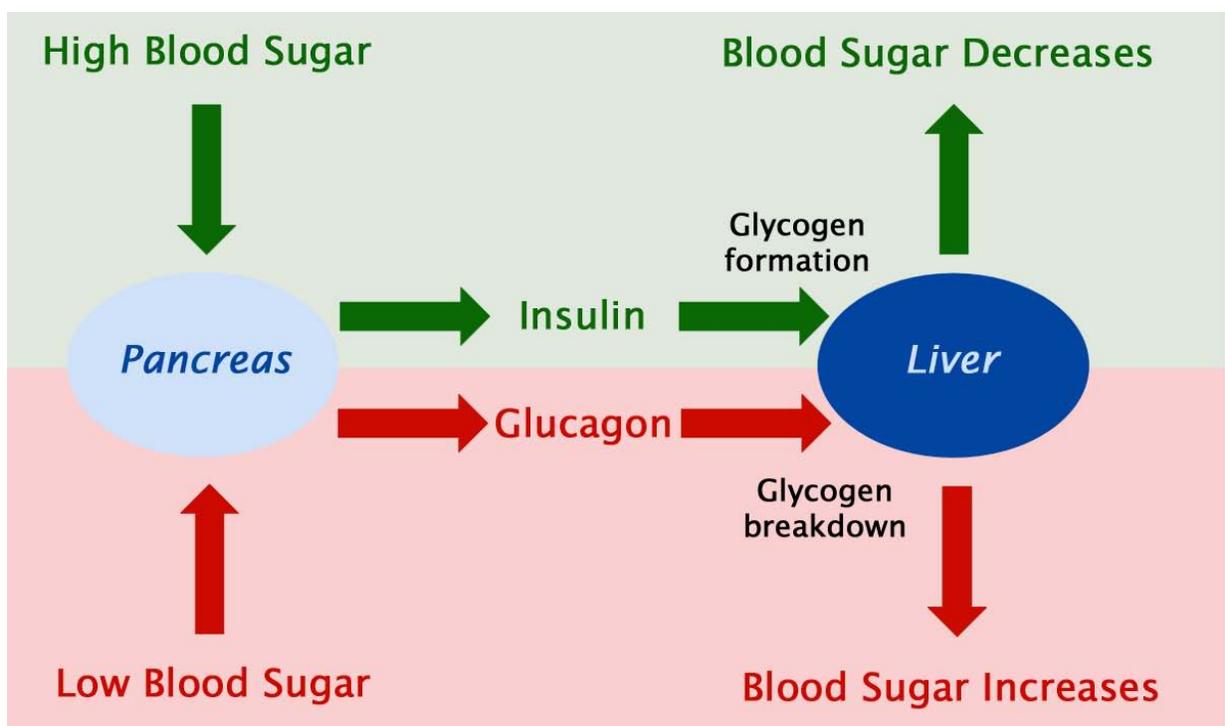


Figure 1: Blood Glucose Energy Regulation by Insulin (top) and Glucagon (bottom).

Cell Biolabs' Human Insulin ELISA Kit is an enzyme immunoassay developed for detection and quantitation of human insulin. The kit utilizes a recombinant human insulin standard and has a detection sensitivity limit of ~50 pg/mL. Each kit provides sufficient reagents to perform up to 96 assays including the standard curve and samples.

Assay Principle

This assay is based on a sandwich ELISA format. Insulin present in samples or standards binds to the anti-insulin antibodies pre-adsorbed on the microtiter plate. Next, a biotinylated anti-insulin antibody is added to the plate well and binds to the captured insulin. A streptavidin-enzyme conjugate is then added, which binds to the biotin of the second antibody. Unbound streptavidin-enzyme conjugate is

removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of insulin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified recombinant human insulin. Sample concentration is then determined by comparing to the known values of the standard curve.

Related Products

1. MET-5030: NAD⁺ / NADH Assay Kit (Fluorometric)
2. MET-5031: NADP⁺ / NADPH Assay Kit (Fluorometric)
3. MET-5051: Human Thyroid-Stimulating Hormone (TSH) ELISA Kit
4. MET-5052: Human Adiponectin ELISA Kit
5. MET-5057: Human Leptin ELISA Kit
6. MET-5062: Human Calcitonin ELISA Kit
7. STA-680: Glucose Assay Kit (Colorimetric)

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Insulin Antibody Coated Plate (Part No. 50631B): One strip well 96-well plate
2. Anti-Insulin Biotinylated Antibody (1000X) (Part No. 50632D): One 10 µL vial of anti-insulin antibody
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL tube
4. Assay Diluent (Part No. 310804): One 50 mL bottle
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle
7. Stop Solution (Part. No. 310808): One 12 mL bottle

Box 2 (shipped on blue ice packs)

1. Insulin Standard (Part No. 50633D): One 10 µL vial of 10 µg/mL human insulin

Materials Not Supplied

1. Insulin samples: human serum, plasma, lysates
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store Insulin Standard at -20°C and avoid freeze/thaw. Store all other components at 4°C.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Insulin Biotinylated Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Anti-Insulin Biotinylated Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.

Preparation of Samples

Samples should be assayed immediately or stored at -80°C prior to performing the assay. Optimal experimental conditions for samples must be determined by the investigator. The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design. A set of serial dilutions is recommended for samples to achieve optimal assay results and minimize possible interfering compounds. Run proper controls as necessary. Always run a standard curve with samples.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80°C for up to three months. Normal plasma samples can be diluted if necessary with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80°C for up to three months. Normal serum samples can be diluted if necessary with 1X PBS containing 0.1% BSA immediately before running the ELISA.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4°C. Assay immediately or store samples at -80°C for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4°C. Assay immediately or store samples at -80°C for up to three months. Dilute samples in 1X PBS containing 0.1% BSA as needed.

Preparation of Standard Curve

1. Prepare fresh standards by diluting the Insulin Standard stock tube from 10 µg/mL to 10 ng/mL (1:1000) in Assay Diluent (Example: Add 2 µL of Insulin Standard stock tube to 1.998 mL of Assay Diluent).
2. Prepare a series of the remaining insulin standards in the concentration range of 3.2 ng/mL – 0.05 ng/mL by diluting the 10 ng/mL according to Table 1 below.

Standard Tubes	10 ng/mL Insulin Standard (µL)	Assay Diluent (µL)	Insulin (ng/mL)
1	320	680	3.2
2	500 of Tube #1	500	1.6
3	500 of Tube #2	500	0.8
4	500 of Tube #3	500	0.4
5	500 of Tube #4	500	0.2
6	500 of Tube #5	500	0.1
7	500 of Tube #6	500	0.05
8	0	500	0

Table 1. Preparation of Insulin Standard Curve.

Note: Do not store diluted insulin standard solutions.

Assay Protocol

Note: Each insulin standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of insulin standards or samples to the Anti-Insulin Antibody Coated Plate. Each sample, standard, blank, and control should be assayed in duplicate.
2. Incubate 1 hour at room temperature on an orbital shaker.
3. Remove the solution from the wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Anti-Insulin Biotinylated Antibody to each well.
5. Incubate 1 hour at room temperature on an orbital shaker.
6. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
7. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
8. Incubate 1 hour at room temperature on an orbital shaker.
9. Remove the solution from the wells. Wash the strip wells 5 times according to step 3 above.
10. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

11. Stop the enzyme reaction by adding 100 μ L of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
12. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Human Insulin ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

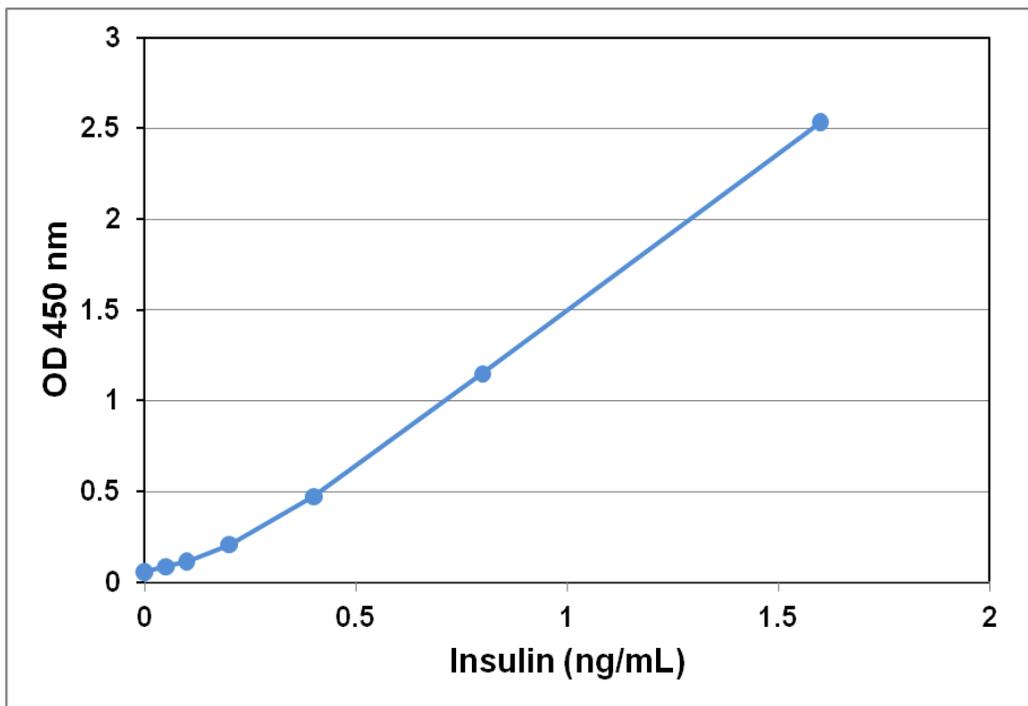


Figure 2: Human Insulin ELISA Standard Curve

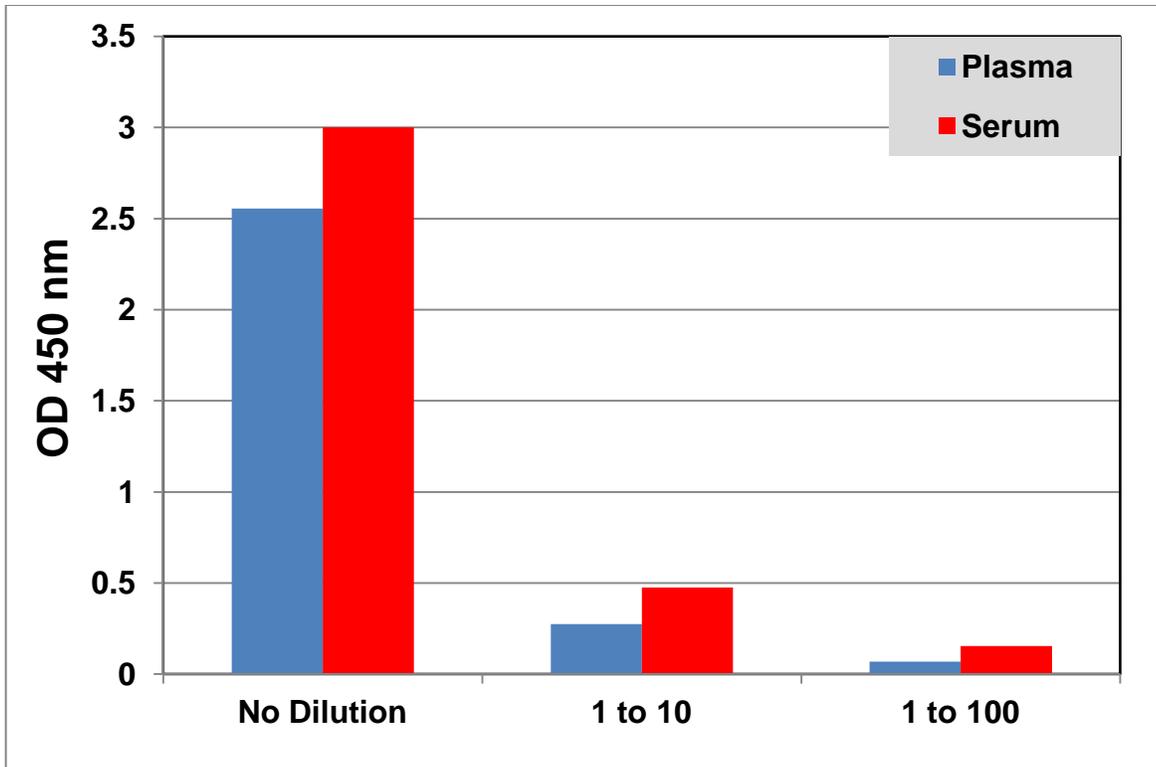


Figure 3: Insulin Levels in Human Plasma and Serum. Human Plasma and Serum were tested undiluted, 1:10, and 1:100 in Assay Diluent according to the Assay Protocol.

References

1. Morgan, C.R., et al. (1863) *Diabetes* **12(2)**: 115-126.
2. Reaven, G.M. (1988) *Diabetes* **37(12)**: 1595-1607.
3. Van den Berghe, G., et al. (2001) *N. Engl. J. Med.* **345**: 1359-1367.
4. Yalow, R.S., et al. (1959) *Nature* **184**: 1648-1649.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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