

ELISA kits available from ADI (see details at the web site)

#0010 Human Leptin
#200-120-AGH Human globular Adiponectin (gAcrp30)
#0700 Human Sex Hormone Binding Glob (SHBG)
#0900 Human IGF-Binding Protein 1 (IGFBP1)
#1000 Human C-Reactive Protein (CRP)
#100-110-RSH Human Resistin /FIZZ3
#100-140-ADH Human Adiponectin (Acrp30)
#100-160-ANH Human Angiogenin
#100-180-APH Human Angiopoietin-2 (Ang-2)
#100-190-B7H Human Bone Morphogenic Protein 7 (BMP-7)
#1190 Human Serum Albumin #1200 Human Albumin (Urinary)
#1750 Human IgG (total) #1760 Human IgM
#1800 Human IgE #1810 Human Ferritin
#1210 Human Transferrin (Tf) #0020 Beta-2 microglobulin
#1600 Human Growth Hormone (GH)

#0060 Human Pancreatic Colorectal cancer (CA-242)
#1820 Human Ovarian Cancer (CA125) #1830 Human CA153
#1840 Human Pancreatic & GI Cancer (CA199)
#1310 Human Pancreatic Lipase
#1400 Human Prostatic Acid Phosphatase (PAP)
#1500 Human Prostate Specific Antigen (PSA) #1510 free PSA (fPSA)
#0500 Human Alpha Fetoprotein (AFP)
#0050 Human Neuron Specific Enolase (NSE)

#0030 Human Insulin #0040 Human C-peptide
#0100 Human Luteinizing Hormone (LH)
#0200 Human Follicle Stimulating Hormone (FSH)
#0300 Human Prolactin (PRL)
#0400 Human Chorionic Gonadotropin (HCG) #0410 HCG-free beta

#0600 Human Thyroid Stimulating Hormone (TSH)
#1100 Human Total Thyroxine (T4) #1110 Human Free T4 (fT4)
#1650 Human free triiodothyronine (fT3) #1700 Human T3 (total)

#1850 Human Cortisol #1860 Human Progesterone
#1865 Human Pregnenolone #1875 Human Aldosterone
#1880 Human Testosterone #1885 Human free Testosterone
#1910 Human Androstenedione #1920 Human Estradiol
#1925 Human Estrone #1940 Dihydrotestosterone (DHT)
#1950 Human DHEA-sulphate (DHEA-S)
#3400 Human serum Neopterin

#3000 Human Rheumatoid Factors IgM (RF)
#3100 Human anti-dsDNA
#3200 Anti-Nuclear Antibodies (ANA)

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Instruction Manual No. M-0030-40-1

Mouse Insulin

ELISA Kit Cat. # 0030-40-1, 96 Tests

For Quantitative Determination of
Mouse Insulin in Serum or Plasma



For In Vitro Research Use Only



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Mouse Insulin ELISA KIT # 0030-40-1, Kit Contents

Components	96 tests
Anti-Insulin coated microwell strip plate (96 wells), Cat. # 30-401	1 plate
Mouse Insulin Calibrator A , 5 ml, 0 ug/ml; #30-402A (yellow)	1 vial
Mouse Insulin Calibrators B-F; 1 ml x 5 (lot specific values printed on the vials) #30-402B-F	5 vials
Anti-Insulin-HRP Conj Conc (11X) 1.3 ml, dilute 1:11 with conjugate buffer #30-403	1 vial
HRP Conjugate buffer, 13 ml, #30-404	1 vial
Wash Buffer Conc (20X); 50 ml, dilute 1:20 with distilled water; #30-40-WB	1 bottle
HRP substrate Solution , 22 ml #30-40-TMB	1 bottle
Stop solution, 7 ml, #30-40-ST	1 bottle
Instruction Manual, M - 0 3 0 4 0	1

Intended Use:

ADI's Mouse insulin ELISA kit is for Quantitative Determination of Mouse Insulin In Serum or Plasma, research use only (RUO).

Introduction

Insulin is the principal hormone responsible for glucose metabolism. It is synthesized in the cells of the islets of Langerhans as the precursor, proinsulin, which is processed to form C-peptide and insulin and both are secreted in equimolar amounts into the portal circulation. The mature insulin molecule comprises two polypeptide chains, the A chain (21 amino acids) and the B chain (30 amino acids), which are linked by two inter-chain disulphide bridges. There is, in addition, a single intra-chain disulphide bridge in the A chain. The sequence of insulin is highly conserved in mammalian species, and is homologous with the insulin-like growth factors IGF-I and IGF-II. Secretion of insulin is mainly controlled by plasma glucose concentration and the hormones have a number of important metabolic actions. Its principal function is to control the uptake and utilization of glucose in peripheral tissues via the glucose transporter. This and other hypoglycemic activities, such as the inhibition of hepatic gluconeogenesis and glycogenolysis are counteracted by the hyperglycemic hormones including glucagons, epinephrine (adrenaline), growth hormone and cortisol. Insulin concentrations are severely reduced in insulin-dependent diabetes (IDDM) and some other conditions such as hypopituitarism. Insulin concentrations may be raised in non-insulin-dependant diabetes (NIDDM), obesity, insulinoma and some endocrine dysfunctions such as Cushing's syndrome and Acromegaly. The main clinical utility measurement is in the investigation of hypoglycemia. Insulin assay have been used in the following applications:

1. To assess the residual cell function, especially in newly diagnosed cases of IDDM.
2. As an aid to the discrimination between IDDM and NIDDM.
3. The diagnosis of insulinoma.
4. In the investigation of the pathophysiology of diabetes mellitus.

Insulin unit conversion

1 ug=174 pmol

PERFORMANCE CHARACTERISTICS

DETECTION LIMIT - Based on sixteen replicates determinations of the zero standards, the minimum concentration of human Insulin detected using this assay is ~ 0.2 ug/L. The detection limit is defined as the value deviating by 2 SD from the zero standards.

Recovery:

Sample dilution (mean 85%); Dilution (mean 96%)

Hook Effect:

No hook effect with high insulin concn up to 500 ng/ml.

Precision:

Intrassay (2.8-5 CV%);
Inter-assay (3.5-11 CV%).

Species reactivity

Cross reactivity with rat, human, porcine, ovine, and bovine insulin. Other species not tested.

	Insulin (%)	Proinsulin I & II (%)	C-peptide I & II (%)	IGF-I (%)	IGF-II %
Mouse		43, 60	<0.002, <0.002	0.02	0.02
Rat	146	14, 60	14, 60		
Human	195	82			
Porcine	628				
Ovine	256				
Bovine	110				

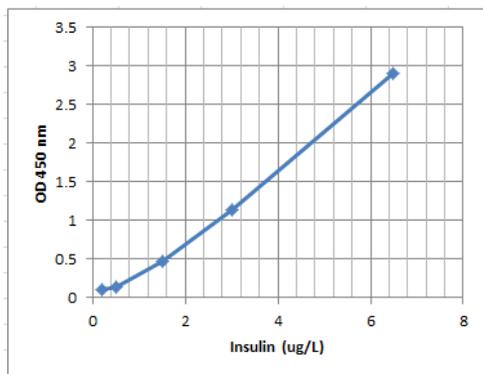
Related Items

Catalog#	ProdDescription
0010	Human Leptin ELISA Kit, 96 tests, Quantitative, 96 tests, Quantitative
0020	Human Beta-2 microglobulin (B2M) ELISA Kit, 96 tests, Quantitative
0030-10-B1	Bovine Insulin ELISA Kit, 96 tests, Quantitative, 96 tests, Quantitative
0030-20-1	Human Insulin-Biotin ELISA Kit, 96 tests, Quantitative, 96 tests
0030-40-1	Mouse Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-50-1	Rat Insulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-60-1	Mouse/Rat Proinsulin ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030-70-1	Mouse/Rat C-Peptide ELISA Kit, High Sensitivity, Quantitative, 96 tests
0030N	Human Insulin ELISA Kit, 96 tests, Quantitative, 96 tests
0035-IA	Human Insulin & Insulin Analogs (Lispro/Humalog, Aspart, Glargine, Glulisine, Detemir) ELISA Kit, 96 tests,
0040	Human C-peptide ELISA Kit, 96 tests, Quantitative

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A _{450nm}	Calculated Conc. (ug/L)
A1, A2	Calib. 0 (0.0 ug/L)	0.072	
B1, B2	Std. B (0.20 ug/L)	0.099	
C1, C2	Std. C (0.50 ug/L)	0.136	
D1, D2	Std. D (1.5 ug/L)	0.466	
E1, E2	Std. E (3 ug/L)	1.136	
F1, F2	Std. F (6.5 ug/L)	2.901	
G1, G2	Sample 1	1.10	1.450

NOTE: These data are for **demonstration purpose only**. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



Kits-spec-XL

A typical std. assay curve (do not use this for calculating sample values)

Calculation of Results

Subtract the absorbance of the zero standard from the mean absorbance values of calibrators and samples.

Plot the A₄₅₀ values of the calibrators against the concentration and use cubic spline regression. For manual plots, read the conc from the calibrator curve.

Insulin assays are the essentials in various dynamic tests, such as oral or intravenous glucose tolerance tests (OGTT and IVGTT), to determine the insulin response of the pancreas and the degree of insulin resistance. In many applications, insulin measurements may be complicated by cross-reactivity with partially degraded insulin, proinsulin and split forms of proinsulin. Immune complexes of these molecules are essentially problematic in patients who have developed anti-insulin antibodies through animal insulin administration.

PRINCIPLE OF THE TEST

Insulin ELISA kit is based on simultaneous binding of Insulin from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of Insulin present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of Insulin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 ul) and Multichannel pipet with disposable plastic tips. Reagent troughs, Plate shaker (orbital shaker), Plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The Alpha Diagnostic Intl., Inc. Insulin ELISA test is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Control Serum has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid). Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H₂SO₄ (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Reagent Preparation:

Dilute wash buffer (1:20) with distilled water (**50 ml** stock buffer and **950 ml** of distilled water). Store at 4oC

Dilute Enzyme conjugate 1:11 with Enzyme conjugate buffer (100 ul stock conjugate and 1 ml of the buffer). Prepare 1 ml per strip or 10 ml for full plate. Do not keep diluted stock and dilute as needed.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the **serum** by centrifugation at room temperature. Do not heat inactivate the serum.. If sera cannot be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. Citrate, EDTA, heparin **plasma** can be stored at -20oC or stored up to 24-hrs for testing.

Sample Dilution

No dilution is normally required. Sample containing >6.5 ug/L or the highest standards should be diluted 1:5 or 1:10 with calibrator 0. Solution containing azide cannot be used in this kit.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping. Standards are stable for two months at 2-8°C. The unused portions of the standards can be frozen in suitable aliquots for long-term use. Repeated freezing and thawing is not recommended.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Dilute wash buffer (1:20) with distilled water (50 ml stock in 1-L of distilled water). Dilute Antibody-HRP Conjugate (1:11) with HRP Conjugate buffer in required volume.

1. Label or mark the microtiter well strips to be used on the plate.
2. Pipet **10 ul of calibrators** and serum samples into appropriate wells in *duplicate*. Dispense **100 ul** of 1x Antibody-Enzyme Conjugate into each well. Gently mix the samples, cover the plate and incubate at **room temp (20-25°C) for 2 hrs on a plate shaker (600-800 rpm) min.** if plate shaker is not available, plates can be manually mixed 3-4 times during the incubation.
3. Wash the plate **6X** with 1x-wash buffer (**350 ul/wash**). We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing
4. Dispense **200 ul TMB substrate per well.** Mix gently for 5-10 seconds, cover the plate and incubate at room temp for **15 min.** Blue color develops in positive wells.
5. Stop the reaction by adding **50 ul of stop solution** to all wells. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the **absorbance at 450 nm** using an ELISA reader within 30 min.

NOTES- Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C.

Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

EXPECTED VALUES

It is recommended that each laboratory determine its own reference values.

Testing of other Biological Fluids Species Crossreactivity

This kit is primarily designed to test human serum samples. It is possible to use the plasma and other biological fluids. However, the sample volume and dilutions must be adjusted according to the expected concentrations or unknown samples be tested at several dilutions to determine the optimum range.

Crossreactivity of insulin antibodies used in the kit with insulin from other species (human, bovine, monkey etc) has not been established.

SPECIFICITY

There is no cross reactivity with C-peptide at the concentration of 5000 pmo/mL, with intact human proinsulin (biosynthetic) 0.3%. High concentrations of lipid or bilirubin do not interfere in the insulin assay. Purified hemoglobin up to 50 ug/mL does not interfere in the test. No interference for rheumatoid factor or human anti-mouse antibodies (HAMA) was observed.

INTERNAL QUALITY CONTROL

Commercial controls such as ADI's Diabetes Antigen Control Rat/Mouse (L, M, H) and/or internal serum pools with low, intermediate and high insulin concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, dilution and/or reconstitution dates of kit components, OD values for the blank, Calibrators and controls.

References: 1. Clark PMS & Hales CN (1991) Assay of Insulin. In P.C. Pickup and G. Williams eds. Textbook of Diabetes, Vol 1, 335-347, Blackwell Scientific Publications; 2. Clark PMS and Hales CN (1994) How to Measure Plasma Insulin. Diabetes/Metabolism Reviews, 10:79-90; 3. Andersen L, Dinesen B, Jorgensen PN, Poulsen F and Roder MF (1993) Enzyme Immunoassay for Intact Human Insulin in Serum or Plasma. Clin Chem 38:578-582; 4. Volund A (1993) Conversion of Insulin units to SI units. American Journal of Clinical Nutrition 58:714-715