

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

<b>#0010</b>	Human Leptin		
<b>#200-120-AGH</b>	Human globular Adiponectin (gAcrp30)		
<b>#0700</b>	Human Sex Hormone Binding Glob (SHBG)		
<b>#0900</b>	Human IGF-Binding Protein 1 (IGFBP1)		
<b>#1000</b>	Human C-Reactive Protein (CRP)		
<b>#100-110-RSH</b>	Human Resistin /FIZZ3		
<b>#100-140-ADH</b>	Human Adiponectin (Acrp30)		
<b>#100-160-ANH</b>	Human Angiogenin		
<b>#100-180-APH</b>	Human Angiopoietin-2 (Ang-2)		
<b>#100-190-B7H</b>	Human Bone Morphogenic Protein 7 (BMP-7)		
<b>#1190</b>	Human Serum Albumin	<b>#1200</b>	Human Albumin (Urinary)
<b>#1750</b>	Human IgG (total)	<b>#1760</b>	Human IgM
<b>#1800</b>	Human IgE	<b>#1810</b>	Human Ferritin
<b>#1210</b>	Human Transferrin (Tf)	<b>#0020</b>	Beta-2 microglobulin
<b>#1600</b>	Human Growth Hormone (GH)		
<b>#0060</b>	Human Pancreatic Colorectal cancer (CA-242)		
<b>#1820</b>	Human Ovarian Cancer (CA125)	<b>#1830</b>	Human CA153
<b>#1840</b>	Human Pancreatic & GI Cancer (CA199)		
<b>#1310</b>	Human Pancreatic Lipase		
<b>#1400</b>	Human Prostatic Acid Phosphatase (PAP)		
<b>#1500</b>	Human Prostate Specific Antigen (PSA)	<b>#1510</b>	free PSA (fPSA)
<b>#0500</b>	Human Alpha Fetoprotein (AFP)		
<b>#0050</b>	Human Neuron Specific Enolase (NSE)		
<b>#0030</b>	Human Insulin	<b>#0040</b>	Human C-peptide
<b>#0100</b>	Human Luteinizing Hormone (LH)		
<b>#0200</b>	Human Follicle Stimulating Hormone (FSH)		
<b>#0300</b>	Human Prolactin (PRL)		
<b>#0400</b>	Human Chorionic Gonadotropin (HCG)	<b>#0410</b>	HCG-free beta
<b>#0600</b>	Human Thyroid Stimulating Hormone (TSH)		
<b>#1100</b>	Human Total Thyroxine (T4)	<b>#1110</b>	Human Free T4 (ft4)
<b>#1650</b>	Human free triiodothyronine (ft3)	<b>#1700</b>	Human T3 (total)
<b>#1850</b>	Human Cortisol	<b>#1860</b>	Human Progesterone
<b>#1865</b>	Human Pregnenolone	<b>#1875</b>	Human Aldosterone
<b>#1880</b>	Human Testosterone	<b>#1885</b>	Human free Testosterone
<b>#1910</b>	Human Androstenedione	<b>#1920</b>	Human Estradiol
<b>#1925</b>	Human Estrone	<b>#1940</b>	Dihydrotestosterone (DHT)
<b>#1950</b>	Human DHEA-sulphate (DHEA-S)		
<b>#3400</b>	Human serum Neopterin		
<b>#3000</b>	Human Rheumatoid Factors IgM (RF)		
<b>#3100</b>	Human anti-dsDNA		
<b>#3200</b>	Anti-Nuclear Antibodies (ANA)		

Instruction Manual No. M-0035-IA

## Human Insulin & Insulin Analogs (Lispro, Aspart, and Glulisine)

### ELISA Kit Cat. # 0035-IA



For Quantitative Determination of  
Human Insulin & Insulin Analogs in Serum or Plasma

*For In Vitro Research Use Only*



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## Human Insulin Analogs ELISA KIT # 0035-IA, Kit Contents

Components	96 tests
Anti-Insulin <b>coated microwell</b> strip plate (96 wells), Cat. # 35-01P	1 plate
Human Insulin <b>Calibrator 0</b> , 5 ml, 0 mU/L; #35-02A (yellow color)	1 vial
Human Insulin <b>Calibrators 1-4; 1 ml x 4 (values stated on the vials) #3502B-E</b>	5 vials
Anti-Insulin-HRP <b>Conj Conc (11X) 600 µl</b> , dilute 1:11 with conjugate buffer #35-03	1 vial
HRP <b>Conjugate buffer</b> , 6 ml, #35-04	1 vial
<b>Wash Buffer</b> Conc (21X); 50 ml, dilute 1:21 with distilled water; #35-WB	1 bottle
HRP substrate <b>Solution</b> , 22 ml #35-TMB	1 bottle
<b>Stop solution</b> , 7 ml, #35-ST	1 bottle
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### Intended Use

ADI's Insulin Analogs (Lispro, Humalog, Aspart, Glargine, Glulisine, Detemir) ELISA kit is a highly sensitive sandwich type assay for the measurement of Insulin or its analog in serum or plasma. For research use only (RUO), not for diagnostic procedures.

### 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<sup>1, 2</sup> 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.

## PERFORMANCE CHARACTERISTICS

**DETECTION LIMIT** -. The detection limit is 1 mU/L calculated as two standard deviations above the calibrator 0.

### Recovery

Recovery upon addition is 101 %

### Hook effect

Samples with a concentration of up to 2000 mU/L can be measured without giving falsely low results.

### Precision:

Sample	Mean mU/L	Within Assay %	%COV	Total assay %
1	15.9	3.0	3.9	4.9
2	53.2	2.8	3.0	4.1
3	90.9	3.2	3.0	4.4

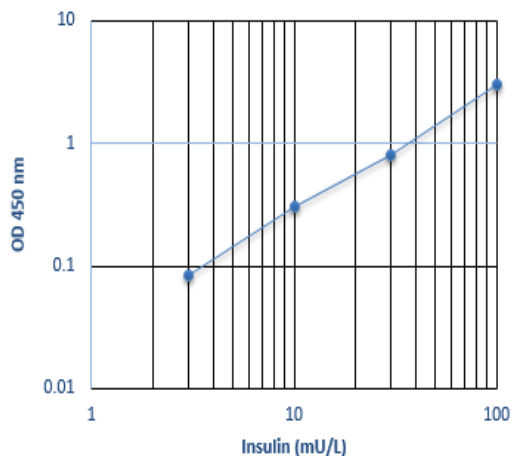
### Reactivity with Insulin analogs and species crossreactivity

Species	% reactivity
Insulin	100%
Insulin lispro	89%
Insulin aspart	80%
Insulin detemir	22%
Insulin glargin	44%
Insulin glulisine	100%
C-peptide	< 0.1%
Proinsulin	54%
Proinsulin des (31-32)	58%
Proinsulin split (32-33)	56%
Proinsulin des (64-65)	66%
Proinsulin split (65-66)	78%
IGF-I	< 0.02%
IGF-II	< 0.02%
Rat insulin	71%
Mouse insulin	49%
Porcine insulin	306%
Ovine insulin	131%
Bovine insulin	58%

## WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean A <sub>450nm</sub>	Calculated Conc. (mU/ml)
A1, A2	<b>Cal 0</b> (0.0 mU/L)	0.061	
B1, B2	<b>Cal. 1</b> (3 mU /L)	0.085	
C1, C2	<b>Cal. 2</b> (10 mU /L)	0.204	
D1, D2	<b>Cal. 3</b> (30 mU /L)	0.791	
E1, E2	<b>Cal. 4</b> (100 mU /L)	2.954	
G1, G2	<b>Sample 1</b>		

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.



Kit-spec-2015-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 A<sub>450</sub> 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), H<sub>2</sub>SO<sub>4</sub> (stop solution), and Proclin-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).

### Reagent Preparation

**Dilute wash buffer (1:21)** with distilled water (**50 ml** stock buffer and **1-L** of distilled water). Store at 4°C

**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 11 ml for full plate. Do not keep diluted stock and dilute as needed.

### SPECIMEN COLLECTION AND HANDLING:

**Serum:** Collect blood by venipuncture; allow clotting, and separating the **serum** by centrifugation at room temperature. If sera cannot be immediately assayed, store samples at **-20°C** for up to six months. Avoid repeated freezing and thawing of samples.

**Plasma:** Collect blood by venipuncture into tubes containing **heparin, citrate or EDTA** as anticoagulant, & separate the plasma fraction. Store samples at **-20°C**, Avoid repeated freezing and thawing of samples.

Samples containing >100 mU/L should be diluted (1:5-1:10) with **calibrator 0**.

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## 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 (1:21) 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 **25 ul of calibrators** and serum samples into appropriate wells in *duplicate*. Dispense **50 ul** of diluted Antibody-Enzyme Conjugate into each well. Gently mix the samples, cover the plate and incubate at **room temp (18-20°C) for 1 hrs on a plate shaker (700-900 rpm)**. 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.
5. 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.
6. 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.. The following results may serve as a guide until the laboratory has gathered sufficient data of its own. Mean fasting levels for 137 tested, apparently healthy individuals, were 10 mU/L, a median of 7 mU/L and a range, corresponding to the central 95% of the observations, of 2–25 mU/L.

## 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 human insulin antibodies used in the kit with insulin from other species (mouse, rat, and monkey) 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.

**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