Catalog #90095

V.5

Insulin ELISA
Kit Instructions

For the quantitative determination of insulin in
human serum and plasma

Catalog #90095
Or #90096 (10 Kit Pack)

96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

Catalog #90095

TABLE OF CONTENTS

A. Intended Use

The Insulin ELISA kit is for the quantitative determination of insulin in human serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Insulin is the primary hormone produced in the ß cells of the islets of Langerhans, and is known not only to regulate glucose metabolism, i.e. the uptake of blood glucose to the liver and peripheral tissues, but also play other important physiological roles.

Recent increases in the incidence of diabetes and obesity have stimulated intensive research on insulin levels and production. As a result, the accurate measurement of insulin and insulin analogues is becoming increasingly important.

C. Principle of the Assay

The Insulin ELISA kit is an ELISA sandwich assay for insulin. It utilizes a specific antibody immobilized onto the microplate wells and an antibody labeled with HRP enzyme. The sample is incubated in the microplate well with HRP labeled antibody. After incubation, all unbound labeled antibody is removed via a wash step. Subsequently, substrate solution is added and insulin levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Insulin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-6	Standards (Lyophilized)	6 x 1 mL
CON1-2	Controls (Lyophilized)	2 x 1 mL
HRP	HRP Labeled Antibody	1 x 1 mL
DIL	Diluent	1 x 11 mL
WASH	Wash Buffer (30X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized water
Microplate sealers
Polypropylene microtubes
Volumetric flasks
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (25 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Serum and plasma samples collected with EDTA or Heparin anticoagulant can be used. Severely hemolyzed samples should be avoided. Samples can be capped and stored at 2-8°C for up to 24 hours prior to assaying. For longer term storage, samples should be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Thawed samples should be inverted several times prior to testing.

I. Assay Procedure

All reagents, unless otherwise noted, are stable for a minimum of 2 months at 2-8°C once opened.

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-6
   Standards are provided in lyophilized form with concentrations ranging from 0 mU/L to 220 mU/L. Dilute each standard with 1 mL of deionized water. After reconstitution, it is recommended that standards be allowed to sit for 5 mins at room temperature and then mixed gently to ensure all solids are dissolved. Reconstituted standards are stable for two weeks at 2-8°C. For longer term storage, reconstituted standards should be frozen. Standards should be not be repeatedly thawed, so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. Standards are approximately provided in the following concentrations: 0, 3, 10, 30, 110 and 220 mU/L. For exact values, please see calibrator labels on each bottle as values may vary slightly from lot to lot.

3. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute each control with 1 mL of deionized water. After reconstitution, it is recommended that controls be allowed to sit for 5 mins at room temperature and then mixed gently to ensure all solids are dissolved. Reconstituted controls are stable for two weeks at 2-8°C. For longer term storage, reconstituted controls should be frozen. Controls should be not be repeatedly thawed, so controls should be appropriately aliquoted in appropriate volumes prior to being frozen.
4. HRP Labeled Antibody
   The HRP labeled antibody must be diluted 1:12 with diluent prior to use. Please prepare only as needed. For example, mix 0.1 mL of HRP labeled antibody with 1.1 mL of Diluent and mix thoroughly. The stability of the working HRP labeled antibody is two weeks when stored at 2-8°C.
5. Diluent
   Provided as ready to use.
6. Wash Buffer (30X Concentrated)
   The wash buffer has to be diluted 1:30 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 1,450 mL of deionized water. Wash buffer is stable for 4 weeks at 2-8°C after dilution, so dilute only as needed.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

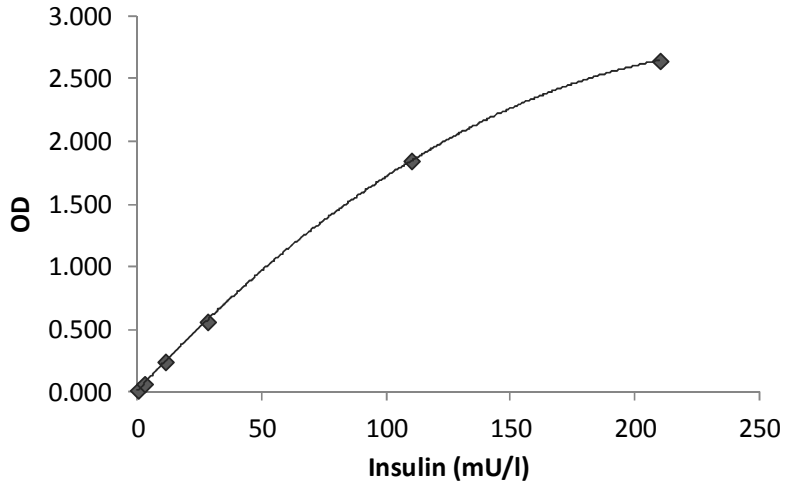
Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling. Remove desired number of coated well strips and add to plate frame. Store remaining strips.

1. In each well, add 100 µL of working HRP labeled antibody into each well.
2. In each well, add 25 µL of sample, standard, or control and mix well by repeated pipetting.
3. Cover the wells with a plate sealer and incubate the plate for 2 hours at 37°C.
4. Aspirate well contents and wash three times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
5. Add 100 µL of Substrate Solution in each well.
6. Incubate the plate for 15 mins in dark room at room temperature.
7. Stop the reaction by adding 100 µL of Stop Solution.
8. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.3. Determining the insulin concentration

1. Using computer software, construct the insulin calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding insulin concentration on the X axis. A four parameter or cubic spline curve fit are suitable for the evaluation. A typical standard curve is shown in Figure 1.

FIGURE 1 Typical Standard Curve



Note: *This curve just an example, and it should not be used to calculate results. A calibration curve should be plotted every time the assay is performed.*

2. Insulin concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor to obtain the final insulin concentration. The insulin concentration is expressed in mU/L.
   Note: *Samples with high insulin concentrations (ie. fall above the range of the assay) should be further diluted with the Standard 1 and rerun.*

J. Performance characteristics

J.1. Assay range

The Insulin ELISA Kit has an assay range from 0.9 – 220 mU/L. The analytical sensitivity of the assay is 0.25 mU/L.

J.2. Precision

The assay has a within-run and total precision of CV $\leq$ 10%.

J.3. Cross reactivity

The table below indicates the analyte and the percent cross reactivity observed in the assay.

TABLE 2 Cross reactivity

Analyte	Cross Reactivity
Insulin	100%
Proinsulin	1.2%
C-Peptide	0%
Insulin Aspart	94%
Insulin Lispro	108%
Insulin Glargine	104%
Mouse Insulin	< 5%
Rat Insulin	< 5%

K. Summary of Insulin ELISA Kit Protocol

Remove desired number of coated well strips and add to plate frame

↓

Add 100 µL of HRP labeled antibody to each well

↓

Add 25 µL of sample, standard or control to each well and mix

↓

Incubate for 2 hours at 37°C

↓

Aspirate and wash wells with 300 µL of Wash Buffer (3x)

↓

Add 100 µL of Substrate Solution per well

↓

Incubate for 15 minutes in the dark at room temperature

↓

Add 100 µL of Stop Solution per well

↓

Read plate within 30 mins at A_{450} (subtract A_{630} values)

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.