V.1

Ultra Sensitive Rat Insulin ELISA Kit, 2nd Gen Instructions

For the quantitative determination of insulin in rat serum, plasma, and cell culture media

Catalog #62300
Or #62305 (10 Kit Bundle)

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

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A. Intended Use

The Ultra Sensitive Rat Insulin ELISA Kit, 2nd Generation (2nd Gen) is for the quantitative determination of insulin in rat serum, plasma, and cell culture media. 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 β cells of the islets of Langerhans. It is known to play an important role in regulating glucose metabolism, i.e. the uptake of blood glucose to the liver and peripheral tissues. In addition, insulin plays an important role in other important physiological processes.

Recent increases in the incidence of diabetes and obesity have prompted intensive research on the effects of modulating and monitoring insulin levels and its production. As a result, the accurate measurement of insulin in experimental animals is important.

C. Principles of the Assay

The Ultra Sensitive Rat Insulin ELISA Kit, 2nd Gen is an ELISA sandwich assay for rat insulin that uses only one intermediate wash step. The kit uses a pair of highly specific monoclonal antibodies for rat insulin. One antibody is coated to the surface of the 96-well plate, and the second is labeled with HRP (horseradish peroxidase). To begin, samples and standards are added to each well along with HRP-labeled antibodies. The rat insulin in the samples or standards binds to both the antibodies coated on the well surface and to the HRP-labeled antibodies in solution. The rat insulin gets ‘sandwiched’ between the two antibodies to form a complex on the surface. After incubation, the plate is washed to remove the excess HRP-labeled antibodies.

The amount of rat insulin in each well is then determined by a colorimetric reaction. TMB (3,3',5,5'-tetramethylbenzidine) is added to each well. The HRP bound to the surface as part of the antibody-insulin-antibody complex reacts with the TMB resulting in a color change from clear to blue. The reaction is stopped, and the, now yellow, color is directly proportional to the amount of HRP present, which is proportional to the amount of rat insulin present in the well. By comparing the magnitude of the color change relative to the known standards, the quantity of rat insulin in the sample can be calculated.

D. Kit Storage

1. Upon receipt of the Ultra Sensitive Rat Insulin ELISA Kit, 2nd Gen, 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-well strips)	1 pack
STD0-7	Standards (0, 0.1, 0.2, 0.5, 1.2, 3, 7.2 and 13 ng/mL)	8 x 1 mL
DIL	Assay Diluent	1 x 20 mL
CONJ	Antibody-HRP Conjugate (11X Concentrate)	1 x 0.8 mL
WASH	Wash Buffer (21X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Adhesive covers for plate	2x, adhesive

E.2. Materials to be supplied by user

Micropipettes and disposable tips
Distilled or deionized water
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 or animal 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 (5 µL), pipetting should be done as carefully as possible. A high quality 10 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 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 to ensure consistent time intervals between wells for each process.
5. Do not mix reagents that have different lot numbers.
6. Do not leave the substrate open to ambient light until just prior to use.

H. Preparation of Rat Plasma and Serum

Plasma: Collect blood in a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL) or EDTA (final concentration: 0.1%), and centrifuge at 4°C for 20 min at 2,000 x *g*.

Serum: Collect blood, allow to clot, and centrifuge at 4°C for 20 min at 2,000 x *g*.

Note: *Be sure to avoid hemolysis during preparation. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged to produce a clear solution. Samples which need to be diluted must be diluted using the Assay Diluent (marked "DIL").*

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate (12 x 8-well strips)
   Provided as ready to use.
2. Standards 0-7
   Provided in liquid, ready to use format ranging from 0 ng/mL to 13 ng/mL. Standards are provided in the following concentrations: 0, 0.1, 0.2, 0.5, 1.2, 3, 7.2, and 13 ng/mL.
3. Assay Diluent
   Provided as ready to use.
4. Antibody-HRP Conjugate (11X Concentrate)
   The Antibody-HRP Conjugate has to be diluted 1:11 with assay diluent just prior to use. Prepare only enough 1X conjugate as needed. For a whole plate, add 700 µL of concentrate to 7 mL of assay diluent or for 6 strips add 350 µL to 3.5 mL. Gently mix to ensure homogeneity. The working HRP solution should be used shortly after it is prepared.
5. Wash Buffer (21X Concentrate)
   The Wash Buffer has to be diluted 1:21 with distilled or deionized water prior to use. Crystal formation in the concentrate is not uncommon when stored at low temperatures. Accordingly, the wash buffer concentrate should be allowed to sit at room temperature (or gently warmed) for at least 30 minutes (and then mixed gently to allow the crystals to fully dissolve). Once any crystals present have dissolved, prepare enough 1X wash buffer as needed. To prepare wash buffer for the full plate, 50 mL of Wash Buffer must be diluted with 1,000 mL of distilled or deionized water. The prepared wash buffer is stable for at least 5 days at 2-8°C.
   Note: *It is not necessary to have the undiluted wash buffer ready prior to starting the assay as it is only needed later in the procedure. We recommend you remove the concentrate from the fridge when you start the assay to provide adequate time for the concentrate to warm to room temperature prior to diluting.*
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

Prior to running the assay or opening any containers, all reagents should be brought to room temperature (20–22 °C) for at least 30 minutes. Mix the reagents thoroughly by gentle agitation or swirling prior to use, and then return the reagents to 2-8°C after use. All samples should be mixed or vortexed to ensure a homogenous composition. If running a partial plate, remove the desired number of strips, and then return and store the unused strips in the pouch at 2-8°C.

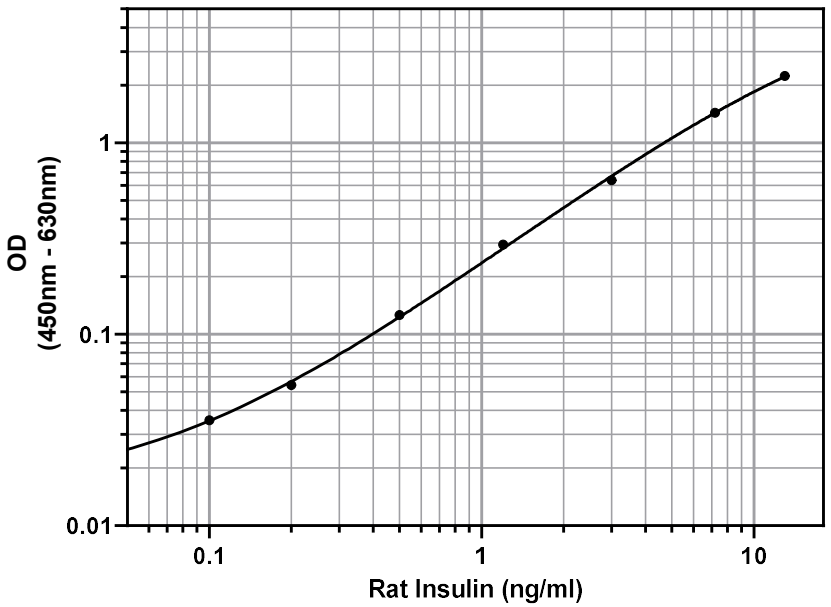
1. In each well, add 50 µL of Assay Diluent.
2. In the appropriate well, add 5 µL of sample or standards.
3. In each well, add 50 µL of 1X antibody-HRP conjugate.
   Note: *Conjugate must be added to all wells, including to the zero standard (0 ng/mL).*
4. Cover the wells with the adhesive plate seal and carefully shake the plate by hand for 15 seconds to mix. Avoid spillage.
5. Incubate the sealed plate for 2.5 hours at room temperature without shaking.
6. Remove the adhesive seal and aspirate well contents. Wash six 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.
7. Add 100 µL of Substrate Solution in each well.
8. Cover the wells with the adhesive plate seal and incubate the plate for 30 mins in the dark at room temperature. Do not use aluminum foil to cover the wells.
9. Add 100 µL of Stop Solution to each well to stop the reaction.
10. Visually inspect the wells to ensure there are no bubbles that will interfere with the assay. Burst any bubbles with a clean pipette tip.
11. Measure the absorbance of each well within 15 minutes at 450nm using a plate reader. If wavelength correction is available on the plate reader, set to 630 nm. If wavelength correction is not available, subtract readings at 630 nm from the readings at 450 nm. This subtraction will correct for any optical imperfections in the plate that might influence absorbance readings.

I.3. Determining the rat insulin concentration

1. Calculate the average absorbance reading for each set of samples or standards.
2. Construct the rat insulin calibration curve by plotting the average absorbance value for each standard on the Y axis versus the corresponding rat insulin concentration on the X axis. Plot the zero standard (0 ng/mL) as part of the curve. To prepare the most accurate curve, use computer software and a 4 or 5 parameter logistic (4PL or 5 PL) curve fit. If a 4PL or 5PL curve cannot be used, a linear regression fit is recommended as an alternative. The data may be linearized by plotting the log of the concentrations on the X axis versus vs. the log of the average ODs on the Y axis and then using regression analysis to determine the best linear fit. This method will produce an adequate but less precise fit of the data.
   Note: *A calibration curve should be plotted every time the assay is performed.*

3. Rat insulin concentrations in the samples are interpolated using the calibration curve. 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 ng/mL. ***Note:*** *Samples with high rat insulin concentrations (ie. fall above the range of the assay) should be diluted with the Assay Diluent and rerun.*

Figure 1: A typical standard curve



J. Performance characteristics

J.1. Assay range and analytical sensitivity

The Rat Insulin ELISA Kit has an assay range from 0.1 – 13 ng/mL. The analytical sensitivity of the assay is ≤ 0.05 ng/mL using a 5 µL sample.

J.2. Precision

The assay has a within-run and total precision of CV ≤ 10%.

Warranty

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Summary of Ultra Sensitive Rat Insulin ELISA Kit, 2nd Gen

Remove the Microplate (marked "MIC") from the pouch.
↓
Dispense 50 µL of Assay Diluent (marked "DIL") per well.
↓
Pipette 5 µL of the sample (or ready-to-use rat insulin standard) per well.
↓
Dispense 50 µL of 1X antibody-HRP conjugate per well.
↓
Cover wells with plate seal, and then gently shake the plate to mix. Incubate the microplate for 2.5 hours at room temp.
↓
Wash each well six times with 300uL wash buffer.*
↓
Dispense 100 µL of Substrate Solution (marked "SUB") per well.
↓
Cover wells with plate seal and incubate the microplate for 30 mins at room temp while avoiding exposure to light.
↓
Dispense 100 µL of Stop Solution (marked "STOP") per well.
↓
Measure absorbance within 15 mins at 450nm and subtract 630nm values.
↓
Calculate insulin concentrations using the standard curve.

* For optimal results, tap the empty plate firmly on a paper towel after each wash step to ensure all buffer is removed.

HIGHLIGHTS

➔ Wide dynamic range of 0.1 – 13 ng/mL using only a 5 µL sample
➔ Easy-to-use procedure requiring only one wash step
➔ High sensitivity of ≤ 0.05 ng/mL using only a 5 µL sample
➔ Ready-to-use liquid standards