Mouse/Rat Insulin

(Ultra sensitive) ELISA Kit

Enzyme Immunoassay for the quantitative determination of Mouse/Rat Insulin in serum, plasma and cell culture supernatants.

Catalog number: ARG81295

Package: 96 wells

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

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

After removal of the precursor signal peptide, proinsulin is post-translationally cleaved into three peptides: the B chain and A chain peptides, which are covalently linked via two disulfide bonds to form insulin, and C-peptide. Binding of insulin to the insulin receptor (INSR) stimulates glucose uptake. A multitude of mutant alleles with phenotypic effects have been identified. There is a read-through gene, INS-IGF2, which overlaps with this gene at the 5' region and with the IGF2 gene at the 3' region. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Jun 2010]

Insulin decreases blood glucose concentration. It increases cell permeability to monosaccharides, amino acids and fatty acids. It accelerates glycolysis, the pentose phosphate cycle, and glycogen synthesis in liver. [provided by Uniprot]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Insulin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Insulin present is bound by the immobilized antibody. After washing away any unbound substances, a HRP (Horseradish Peroxidase)-conjugated antibody specific for Insulin is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Insulin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Insulin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	2 x 6 strips x 8-well	4°C
Standards (2.56 ng)	1 vial	4°C
Sample/Standard Diluent	30 ml (ready-to-use)	
HRP-anti-Insulin Conjugate (concentrate)	8 ml	4°C
Conjugated Antibody Diluent	4 ml (ready-to-use)	4°C
20X Wash Buffer	50 ml	4°C
TMB substrate	13 ml (ready-to-use)	4°C (Protect from light)
STOP solution	13 ml (ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 630 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex mixer
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 2-8°C and avoid light exposure at all times.
- Briefly spin down the reconstituted **S**tandard before use.
- If crystals are observed in the 20X Wash buffer, warm to RT until the crystals are completely dissolved.
- In order to prevent the microplate wells from drying out, samples and reagents should be dispensed quickly into the wells. In no case should 10 min be exceeded per plate per pipetting step.
- The wash procedure should be done thoroughly in order to minimize background readings.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards and samples be assayed in duplicates.
- Avoid using reagents from different batches.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernatants - Remove particulates by centrifugation at 2-8 °C and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum- Use a serum separator tube (SST) and allow samples to clot before centrifugation. Centrifuge samples (2000 x g) for 20 minutes at 2-8 °C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using heparin (final concentration at 1 unit/ml), EDTA (final concentration at 0.1%), or sodium citrate (final concentration at 0.76%) as an anticoagulant. Centrifuge (2000 x g) for 20 minutes at 2-8 °C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Avoid using hemolysis samples. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged to produce a clear solution.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for one week at 2-8°C. Prepare only enough as needed.
- Working HRP-anti-Insulin Conjugate solution: For 48 wells used, prepare the needed volume of working HRP-anti-insulin conjugate solution by mixing 3.6 ml of HRP-Anti-Insulin Enzyme Conjugate (concentrate) with 1.8 ml of Conjugated Antibody Diluent, and mix completely to ensure a homogeneous and clear solution. Prepare only enough as needed. Avoid foaming during mixing.

  Note: The working HRP-anti-insulin enzyme conjugate solution should be prepared just before the second reaction and must be used immediately
- Sample:

  a) If in an initial assay, a specimen is found to contain insulin concentration more than the highest standard, the specimens can be diluted with Sample/Standard Diluent and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

  b) If the specimen believed to contain an insulin lower than the lowest standard (0.1 ng/ml), the tested sample volume can be increased from 5 µl to a maximum of 100 µl to provide increased sensitivity. Using a 100 µl sample, the low range assay can detect a minimum insulin concentration of 5 pg/ml.

- **Standards**: Reconstitute the standard with **100 µl** of **distilled water** to yield a stock concentration of **25.6 ng/ml**. Allow the stock standard to sit for few minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. The reconstituted mouse insulin stock solution can be stock at 2-8°C up to one week or at -20°C up to one month. For longer storage, stock solution should be stored at-80°C.

  The Sample/Standard diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **6.4 ng/ml, 3.2 ng/ml, 1.6 ng/ml, 0.8 ng/ml, 0.4 ng/ml, 0.2 ng/ml, 0.1 ng/ml.**

Dilution table for Human Insulin standard preparation:

Insulin Concentration (ng/ml)	Volume of Sample/Standard diluent (µl)	Volume of standard (µl)
6.4	150	50 (25.6 ng/ml)
3.2	50	50 (6.4 ng/ml)
1.6	50	50 (3.2 ng/ml)
0.8	50	50 (1.6 ng/ml)
0.4	50	50 (0.8 ng/ml)
0.2	50	50 (0.4 ng/ml)
0.1	50	50 (0.2 ng/ml)
0	50	

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use and should be stored at 2-8°C immediately after use. Before use, mix the reagents and samples thoroughly by gentle agitation or swirling. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.

For 5 µl samples

2. Add **95 µl** of **Sample/Standard diluent** in each well.
3. Add **5 µl standards and samples** in duplicate into the appropriate wells.
4. Cover the microplate with the plastic microplate cover and incubate for **2 hours at 4°C**.

For samples volume >5 µl

2. Add **95 µl** of **Sample/Standard diluent** in the standard wells and add **5 µl standards** in duplicate into the wells.
3. Add **samples** with volume > 5 µl into the appropriate wells, and then add **Sample/Standard diluent** to a total volume of 100 µl.

   For example:

Sample volume (µl)	Sample/Standard diluent (µl)	Total volume (µl)	Calculation Factor
5	95	100	1
20	80	100	4
50	50	100	10
100	0	100	20

4. Cover the microplate with the plastic microplate cover and incubate for **2 hours at 4°C**.
5. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes.** Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
6. Add **100 µl of working HRP-Insulin antibody solution** into each well. Mix thoroughly for 5-10 seconds. Cover the microplate and then **incubate for 30 minutes at RT**.
7. Aspirate each well and wash, repeating the process 6 times for a **total 7 washes.** Wash by filling each well with **1X Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
8. Add **100 µl** of **TMB mixture** to each well. Incubate **for 40 minutes at room temperature** in dark.

   Note: Do not cover the microplate with aluminum foil.
9. Add **100 µl** of **Stop Solution** to each well.
10. Read the OD with a microplate reader at **450 nm** immediately. It is recommended that the wells be read within 30 minutes after adding the Stop Solution. (Optional: read at 630 nm as reference wavelength)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. The mean absorbance of the 0 ng/ml standard should be less than 0.1.

7. For the sample volume > 5µl: The insulin concentration of the sample should be calculated as follows:

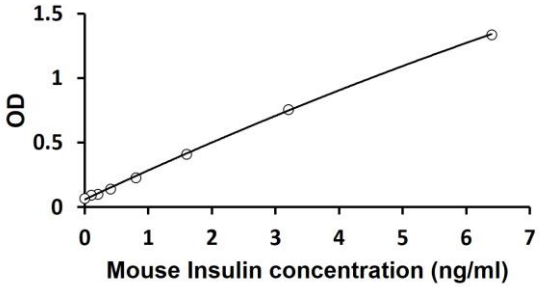
Insulin concentration of sample (ng/ml) =

Calculated Concentration (ng/ml) / Calculation Factor

[Calculation Factor = Adding sample volume/5]

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



The mean O.D. of the zero standard (0 ng/ml) should be <0.1.

QUALITY ASSURANCE

Sensitivity

Minimum Detectable Concentration:

50 pg/ml (5 µl sample volume); 5 pg/ml (100 µl sample volume)

Specificity

Substance	Cross-reactivity (%)
Mouse Insulin	100
Rat Insulin	95
Hamster Insulin	230
Rabbit Insulin	275
Dog Insulin	190
Porcine Insulin	325
Bovine Insulin	220
Recombinant Human Insulin	350

Mouse C-peptide	Not detectable
Rat C-peptide	Not detectable
Rat pancreatic polypeptide	Not detectable
Porcine glucagon	Not detectable
Human insulin like growth factor I	Not detectable
Human insulin like growth factor II	Not detectable

Intra-assay and inter-assay precision

The CV value of intra-assay and inter-assay precisions were ≤ 10%

Recovery

85 - 115%