Human/Canine/Porcine Insulin ELISA Kit

Enzyme Immunoassay for the quantification of Human/Canine/Porcine Insulin in Serum, plasma and cell culture supernatants. (The reactivity to Canine and Porcine insulins are predicted to be worked based on sequence homology.)

Catalog number: ARG82618

Package: 96 wells

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

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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. [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 biotin-conjugated antibody specific for Insulin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. 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	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	2 Vials	4°C
Standard diluent buffer	5 ml	4°C
Antibody conjugate concentrate	1 vial (120 µl)	4°C
HRP-Streptavidin concentrate	1 vial (150 µl)	4°C (Protect from light)
10X Assay buffer	5 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	15 ml	4°C (Protect from light)
STOP solution	15 ml	4°C
Plate sealer	5 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: for wavelength correction, 570nm or 630nm is recommended)
- Pipettes and pipette tips
- Deionized or distilled water
- 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 4°C at all times.
- If crystals are observed in the 20X Wash buffer or 10X Assay buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 15-20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 30-60 sec or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.

- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Once the assay has been started, all subsequent steps should be completed without interruption and within the recommended time limits.
- Avoid using reagents from different batches.
- Take care not to scratch the inner surface of the microwells.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- It is recommended to add 1X assay buffer, samples, controls and 1X Antibody conjugate into wells within 15 min.
- Change pipette tips between the addition of different reagent or samples.

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 for 10 min at 300 x g and aliquot & store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note:

a) Samples containing a visible precipitate must be clarified by centrifuge prior to use in the assay.
b) Do not use haemolytic, icteric or lipaemic specimens.
c) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute **20X** Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 25 ml of 20X Wash buffer + 475 ml of distilled water) The diluted 1X Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Assay buffer**: Dilute **10X** Assay buffer into distilled water to yield 1X Assay buffer. (E.g. 5 ml of 1X Assay buffer + 45 ml of distilled water) The diluted 1X Assay buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate**: Stock reagent should be mixed well prior to making dilutions. 5-10 minutes before use (freshly prepared is recommended), dilute **100X** antibody conjugate concentrate into 1X Assay buffer to yield 1X detection antibody solution, mix well. (e.g. 10 µl of 100X Antibody conjugate concentrate + 990 µl of 1X Assay buffer) The diluted antibody solution must be used within 30 minutes after dilution.
- **1X HRP-Streptavidin Solution**: Stock reagent should be mixed well prior to making dilutions. 5-10 minutes before use (freshly prepared is recommended), dilute **100X** HRP-Streptavidin concentrate solution into 1X Assay buffer to yield 1X HRP-Streptavidin Solution buffer, mix well. (e.g. 10 µl of 100X HRP-Streptavidin concentrate solution + 990 µl of 1X Assay buffer) The diluted HRP-Streptavidin Solution must be used within 30 minutes after dilution.
- **Sample**: Plasma or serum sample should be diluted 1:1 with 1 x Assay Buffer (add sample into equal volume of 1 x Assay Buffer) and mix well prior to assay (dilution factor = 2). Cell culture supernatant samples may be assayed directly. If the measuring absorbance of samples is higher

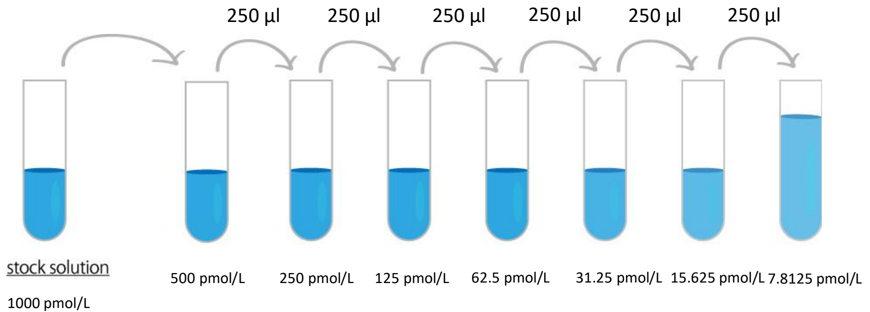
than the highest standard, dilute the serum or plasma samples with 1X Assay buffer before assay; dilute the cell culture samples with cell culture medium and assay again. For the calculation of the concentrations this dilution factor has to be taken into account.

(It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards**: Reconstitute the standard with distilled water (volume is lot dependent) to yield a stock concentration of **1000 pmol/L**. Allow the stock standard to sit for 10-30 minutes with gentle agitation to make sure the standard is dissolved completely before making serial dilutions. Do not induce foaming.

  Standards for serum or plasma samples: Mix 250 µl of the reconstituted standard with 250 µl Standard diluent buffer to yield a highest concentration standard (S7) concentration of 500 pmol/L. The standard diluent buffer serves as zero standard (0 pmol/L), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **500 pmol/L, 250 pmol/L, 125 pmol/L, 62.5 pmol/L, 31.25 pmol/L, 15.625 pmol/L, 7.8125 pmol/L.**

  Standards for cell culture supernatants**:** Mix 250 µl of the reconstituted standard with 250 µl cell culture medium to yield a highest concentration standard (S7) concentration of 500 pmol/L. The cell culture medium serves as zero standard (0 pmol/L), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: **500 pmol/L, 250 pmol/L, 125 pmol/L, 62.5 pmol/L, 31.25 pmol/L, 15.625 pmol/L, 7.8125 pmol/L.**



Dilute Insulin standard as according to the table below:

Standard	Insulin Conc.	µl of diluent	µl of standard
S7	500 pmol/L	250	250 (1000 pmol/L Stock)
S6	250 pmol/L	250	250 (S7)
S5	125 pmol/L	250	250 (S6)
S4	62.5 pmol/L	250	250 (S5)
S3	31.25 pmol/L	250	250 (S4)
S2	15.625 pmol/L	250	250 (S3)
S1	7.8125 pmol/L	250	250 (S2)
S0	0	250	0

ASSAY PROCEDURE

All materials including microplate, samples, standards and working solutions should be equilibrated to room temperature (20-25°C, RT) before use. 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.
2. Wash the plate **once** by filling each well with **1× Wash Buffer** (**300 µl**) using a squirt bottle, manifold dispenser, or autowasher, keep the wash buffer in the wells for 30 sec before remove. 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. DO NOT let the wells completely dry at any time.
3. Add **100 µl** of **samples standards and zero controls** (Standard diluent buffer or cell culture medium) into appropriate wells.
4. Add **50 µl** of **1X Antibody conjugate** into wells. Cover wells and incubate for 2 hour at RT on a microplate shaker set at 300 rpm.
5. Cover wells and incubate for **90 minutes at RT** on a microplate shaker (~300 rpm).
6. Aspirate each well and wash for a **total six washes** as step 2.
7. Add **100 µl** of **1X HRP-Streptavidin solution** to each well. Cover wells and incubate for **30 minutes at RT** on a microplate shaker (~300 rpm).
8. Aspirate each well and wash as step 8 (for a **total six washes)**.
9. Add **100 µl** of **TMB Reagent** to each well. Incubate for **5-30 minutes at RT** in dark.
10. Add **100 µl** of **Stop Solution** to each well. The color of the solution should

change from blue to yellow.

11. Read the OD with a microplate reader at **450 nm** immediately. (optional: read at 570 nm or 630 nm as the reference wave length) It is recommended read the absorbance within 30 minutes after adding the stop solution.

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.

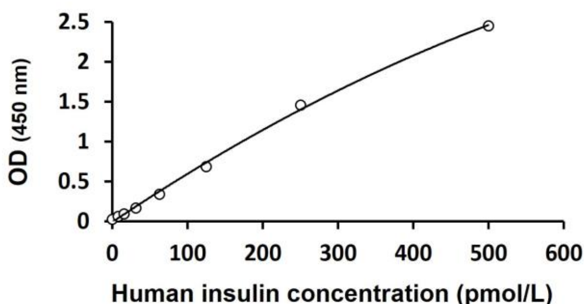
5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

7. Conversion: 1 pg/ml = 5.808 pmol/L. (1 pmol/L = 0.172 pg/ml)

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.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Human/Canine/Porcine Insulin ranged from 7.8- 500 pmol/L. The mean MDD was 3.9 pmol/L.

Specificity

This assay recognizes natural and recombinant Human/Canine/Porcine Insulin. No significant cross-reactivity or interference with the factors below was observed:

Human: IGF1, IGF2, IL2, IL4, IL6, IL8, IL10, IL12, IL18, IL22, Insulin R, MCP1, Relaxin 1, Relaxin 2, Relaxin 3, TGF beta 1, TNF alpha and VEGF.

Mouse: GM-CSF, IFN gamma, IL1 beta, IL2, IL4, IL6, IL10, IL17A and TNF alpha.

Rat: IFN gamma, IL1 beta, IL4, IL6, IL10 and TNF alpha.

Recovery

88-115%

Linearity

83-118%

Intra-assay and Inter-assay precision

The CV values of intra-assay was 4.5% and inter-assay was 4.2%.