V.2

Total Proinsulin ELISA Kit Instructions

For the quantitative determination of total proinsulin in human serum and plasma

Catalog #90110
96 Assays

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

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A. Intended Use

The Total Proinsulin ELISA kit is for the quantitative determination of total proinsulin 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

Proinsulin is synthesized by the β-cell of the pancreas as a precursor molecule for insulin. Normally, proinsulin is rapidly degraded in the bloodstream. An increase in the insulin demand, as provided by insulin resistance in later stages of type 2 diabetes mellitus or in patients with insulinoma, can result in increased expression of proinsulin. Monitoring the total proinsulin values using a highly specific assay in conjunction with intact proinsulin values can provide useful information about how insulin is processed and used to serve as the basis for the selection of an insulin resistance therapy.

C. Principle of the Assay

The Total Proinsulin ELISA kit is an ELISA sandwich assay for total proinsulin and utilizes a specific antibody immobilized onto the microplate wells and a soluable antibody labeled with HRP. In the first step, proinsulin in the sample binds to the antibody coated microtiter plate. Subsequently, HRP labeled antibody is added to bind to the immobilized proinsulin. After incubation, all unbound labeled antibody is removed via a wash step. Finally, substrate solution is added and proinsulin levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Total Proinsulin 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
STD1-5	Standards (Lyophilized)	5 x 1 vial
ConDIL	Conjugate Diluent	1 x 11 mL
HRP	HRP Labeled Antibody	1 x 1mL
BUF	Sample Buffer	1 x 12mL
WASH	Wash Buffer (30X Concentrate)	1 bottle
SUB	Substrate (TMB) Solution	1 bottle
STOP	Stop Solution	1 bottle

E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized water
Microplate sealers
Polypropylene microtubes
Vortex mixer
Microplate reader (capable of reading A_{450} and $A_{620/650}$ 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 (50 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.
6. Avoid microbial contamination to minimize false results.

H. Sample Collection

Plasma samples should be collected with EDTA or heparin anticoagulant and immediately centrifuged after collection. Serum samples should be allowed to clot over 30 minutes and then the clot removed via centrifugation prior to use. Hemolytic samples should be avoided. Samples should be used immediately or stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Thawed samples should be inverted several times prior to testing.

I. Assay Procedure

I.1. Preparation of reagents

Prior to preparing any materials, all reagents should be brought to room temperature before use.

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture. Unused wells are stable for up to 2 months at 2-8°C.
2. Standards 1-5
   Standards are provided in lyophilized form with concentrations ranging from 0.0 to 220 pmol/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 thoroughly, but gently, with a vortex mixer to dissolve all solids. Reconstituted standards are stable for two weeks at 2-8°C.
3. Conjugate Diluent
   Provided as ready to use.
4. HRP Labeled Antibody
   Transfer entire bottle of HRP into the bottle of Conjugate Diluent and mix thoroughly. Use within 24hours.
5. Wash Buffer (30X Concentrated)
   Prepare a working concentration of buffer by diluting 1 part of Wash Buffer with 29 parts of distilled or deionized water. For example, 50 mL of wash buffer must be diluted with 1450 mL of deionized or distilled water. Wash buffer is stable for 2 weeks at 2-8°C after dilution, so dilute only as needed.
6. Substrate Solution
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

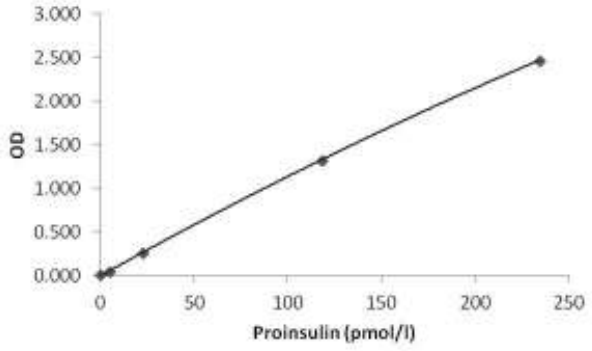
Prior to running the assay, all reagents should be brought to room temperature. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Add 50 µL of Sample Buffer and 50 µL of sample or standard to each well, and mix by repeated pipetting.
2. Seal the plate, and incubate for 2 hours at room temperature.
3. Remove the plate seal, and aspirate well contents. Wash three times using 300 µL of working strength Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of working strength HRP antibody solution in each well, and then gently agitate the plate to mix.
5. Seal the plate, and incubate the plate for 1 hour at room temperature.
6. Remove the plate seal, and aspirate well contents. Wash three times using 300 µL of working strength 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, and then gently agitate the plate the mix.
   Note: Take care not to cross-contaminate the Substrate solution with the HRP solution.
8. Seal the plate, and incubate the plate for 15 minutes at room temperature in the dark.
9. Remove the seal, and stop the reaction by adding 100 µL of Stop Solution to each well.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values with the optical density (OD) normalized by subtracting $A_{620/650}$ values).

I.3. Determining the Total Proinsulin (pmol/L)

1. Using computer software, construct the total proinsulin calibration curve by plotting the mean optical density for each standard on the Y axis versus the corresponding total proinsulin concentration (pmol/L) on the X axis, Figure 1. Using a cubic spline curve fit, sample concentrations can be read directly from the calibration curve. Other types of data processing fit functions may give slightly different results.
   Note: *A calibration curve should be plotted every time the assay is performed.*

Figure 1 – Example Calibration Curve



2. Proinsulin concentrations in the samples are interpolated using the calibration curve and mean optical density values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor to obtain the final proinsulin concentration. The proinsulin concentration is expressed in pmol/L.
   Note: Samples that fall outside the range of the standards should be diluted with sample buffer by a factor and rerun

J. Performance characteristics

J.1. Assay range

The Total Proinsulin ELISA Kit has an assay range from 0.5 – 220 pmol/L.

J.2. Sensitivity

The analytical sensitivity of the assay is 0.5 pmol/L.

J.3. Precision

The asaay has a within-run and total precision of CV <10%, and the table below indicates the analyte and the percent cross reactivity observed in the assay.

TABLE 2 – Cross Reactivity

Analyte	Cross Reactivity (%)
Proinsulin	100%
Insulin	2.2%
C-Peptide	0%
32-33 split proinsulin	97%
Des 31-32 split proinsulin	100%

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