ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Proinsulin ELISA Kit

(Catalog Number: 31420)

For the quantitative determination of human proinsulin concentrations in serum or plasma

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INTRODUCTION

Proinsulin is a polypeptide of 86 amino acids made in the beta cells of the pancreas and is the precursor molecule for insulin, most proinsulin is converted to insulin and C-peptide secreted into the blood. Increased circulating levels of proinsulin are found in patients with insulinomas, hypoglycemia and hyperinsulinemia.

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a monoclonal antibody specific for human proinsulin. Standards and samples are pipetted into the wells and any human proinsulin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin labelled monoclonal antibody specific for human proinsulin is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and color develops in proportion to the amount of human proinsulin bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured human proinsulin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Human Proinsulin ELISA kit is designed for quantification of human proinsulin in serum and plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with a monoclonal antibody against human proinsulin, sealed
2. Sample diluent, 6 mL, ready for use
3. 10×Wash buffer, 50 mL
4. 5×Assay buffer, 10 mL
5. 100×Detection antibody solution, a biotin labelled monoclonal antibody against human proinsulin, 0.12 mL
6. Human proinsulin standard, 2000 pg of recombinant human proinsulin, lyophilized
7. 200×STP-HRP solution, 0.06 mL
8. Substrate solution, 12 mL, ready for use
9. Stop solution, 12 mL, ready for use
10. Plate cover

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper

5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the human proinsulin microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (20 mL) with 80 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (50 mL) with 450 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

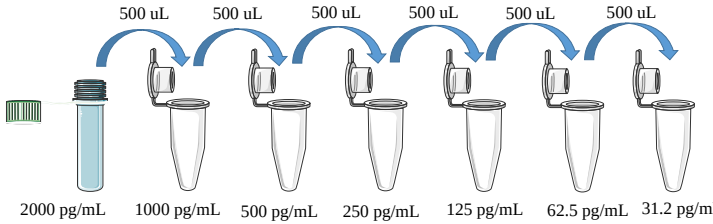
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required per well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS

Human Proinsulin Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 2000 pg/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 1000, 500, 250, 125, 62.5, 31.2 pg/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -80ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 50 µL of Sample diluent to each well.
2. Add 50 µL of standard or sample per well. Seal the plate with a plate cover. Incubate at room temperature for 1 hour, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
3. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
4. Add 100 µL of 1×Detection antibody solution to each well. Seal the plate with a plate cover. Incubate at room temperature for 1 hour, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
5. Wash each well 3 times as in step 2.
6. Add 100 µL of 1×STP-HRP solution to each well. Seal the plate with a plate cover. Incubate at room temperature for 20 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
7. Wash each well 4 times as described in step 2.
8. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light**.
9. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
10. Measure absorbance of each well at 450 nm immediately.

CALCULATION

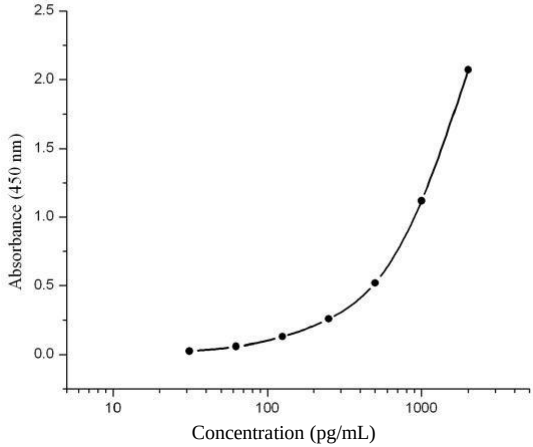
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human proinsulin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human proinsulin concentration of samples from standard curve.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Human proinsulin (pg/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.096	0
31.2	0.122	0.026
62.5	0.154	0.058
125	0.225	0.129
250	0.354	0.258
500	0.616	0.52
1000	1.214	1.118
2000	2.168	2.072

Human proinsulin standard (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of proinsulin that can be measured by this assay is 31.2 pg/mL.

B. Precision

Intra-assay Precision (Precision within an assay) C.V. <6.5%.
Inter-assay Precision (Precision between assays) C.V. <7.3%.

C. Recovery
The average recovery was 89.9%.

D. Linearity
98-113%.

SUMMARY OF ASSAY PROCEDURE

Add 50 µL of Sample diluent to each well.
↓
Add 50 µL of standard or sample to each well.
↓
Incubate at room temperature for 1 hour (600 rpm).
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour (600 rpm).
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes (600 rpm).
↓
Aspirate and wash each well four times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation