

Human Proinsulin immunoassay kit

Catalogue Number: 31420

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

This package insert must be read in its entirety before using this product.

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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 ELISA. The immunoplate 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-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human proinsulin bound initially. The assay is stopped and the optical density of the wells 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. Micro-titre 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 in buffered protein base, lyophilised.
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-3.

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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 microplate, 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 l 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 l 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 AND SAMPLES

Human proinsulin standards: Reconstitute the lyophilised 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. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
2000 pg/ml stock	-	2000 pg/ml
250 l of 2000 pg/ml	250 l	1000 pg/ml
250 l of 1000 pg/ml	250 l	500 pg/ml
250 l of 500 pg/ml	250 l	250 pg/ml
250 l of 250 pg/ml	250 l	125 pg/ml
250 l of 125 pg/ml	250 l	62.5 pg/ml
250 l of 62.5 pg/ml	250 l	31.2 pg/ml

1×Assay buffer serves as the zero standard (0 pg/ml).

The reconstituted standard stock should be aliquoted and stored at -80°C 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 should be assayed in duplicate.

1. Add 50 μ l of Sample diluent to each well.
2. Add 50 μ l 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 μ l of 1 \times Wash buffer to each well and incubate for 1 minute. Discard the 1 \times 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 μ l of 1 \times 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 μ l of 1 \times 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 μ l of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
9. Add 100 μ l 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: 89.9%

D. Linearity: 98-113%

SUMmary OF Assay procedure

Add 50 μ l of Sample diluent to each well.

Add 50 μ l 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 μ l of 1 \times Detection antibody solution to each well.

Incubate at room temperature for 1 hour (600 rpm).

Aspirate and wash each well three times.

Add 100 μ l of 1 \times STP-HRP solution to each well.

Incubate at room temperature for 20 minutes (600 rpm).

Aspirate and wash each well four times.

Add 100 μ l of Substrate solution to each well.

Incubate at room temperature for 15 minutes.

Add 100 μ l of Stop solution to each well.

Measure absorbance of each well at 450 nm.

Calculation