Human C Peptide ELISA Kit

Enzyme Immunoassay for the quantification of C Peptide in serum, plasma and urine

Catalog number: ARG80782

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INTRODUCTION

Insulin is synthesized in the pancreatic beta cells as a 6000 MW component of an 86 amino acid polypeptide called proinsulin. Proinsulin is subsequently cleaved enzymatically, releasing insulin into the circulation along with a residual 3000 MW fragment called connection ("C") peptide, so named because it connects A and B chains of insulin within the proinsulin molecule. Human C-Peptide, a 31 amino acid residue peptide, has a molecular mass of approximately 3000 daltons. C-Peptide has no metabolic function. However, since C-Peptide and insulin are secreted in equimolar amounts, the immunoassay of C-Peptide permits the quantitation of insulin secretion. This is the reason for the clinical interest of serum and urinary determinations of C-Peptide. Moreover, C-Peptide measurement has several advantages over immunoassays of insulin. The half-life of C-Peptide in the circulation is between two and five times longer than that of insulin. Therefore, C-Peptide levels are a more stable indicator of insulin secretion than the more rapidly changing levels of insulin. A very clear practical advantage of C-Peptide measurement arising from its relative metabolic inertness as compared to insulin is that C-Peptide levels in peripheral venous blood are about 5-6 times greater than insulin levels. Also, relative to an insulin assay, the C-Peptide assay's advantage is its ability to distinguish endogenous from injected insulin. Thus, low C-Peptide levels are to be expected when insulin is diminished (as in insulin-dependent diabetes) or suppressed (as a normal response to exogenous insulin), whereas elevated C-Peptide levels may result from the increased β-cell activity observed in insulinomas. C-Peptide has also been measured as an

additional means for evaluating glucose tolerance and glibenclamide glucose tests. C-Peptide levels are in many ways a better measurement of endogenous insulin secretion than peripheral insulin levels. C-Peptide may be measured in either blood or urine. With improved sensitive C-Peptide immunoassays, it is now possible to measure C-Peptide values at extremely low levels. The clinical indications for C-Peptide measurement include diagnosis of insulinoma and differentation from factitious hypoglycemia, follow-up of pancreatectomy, and evaluation of viability of islet cell transplants. Recently, these indications have been dramatically expanded to permit evaluation of insulin dependence in maturity onset diabetes mellitus.

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of C-Peptide in serum, plasma and urine. This assay employs the competitive quantitative enzyme immunoassay technique. A specific C-Peptide monoclonal antibody has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and C-peptide in the samples are bound by the immobilized antibodies. After washing, a fixed amount of C-peptide labeled with biotin compete with unlabeled C-peptide binding sites on the monoclonal antibodies. HRP is then incubated and bound to biotin-labeled C-peptide. After washing away any unbound substances, a substrate solution (TMB) is then added to the wells and color develops in inverse proportion to the amount of C-peptide 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 450 nm ±2 nm. The concentration of C-peptide 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	12 X 8 strips	4°C
Standard 0-5	6 vials (Lyophilized) Add 0.75 ml distilled water to reconstitute	4°C
Sample Diluent	1 vial (3ml) (Ready-to-use)	4°C
Antiserum	27ml (Ready-to-use)	4°C
C-peptide Biotin	14ml (Ready-to-use)	4°C
HRP-Conjugate	14ml (Ready-to-use)	4°C
40X Wash buffer	30 ml	4°C
TMB substrate	14 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	14 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- 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.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- 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.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

Urine – Aliquot a well-mixed sample to be used in the assay. Centrifuge samples to clear. Urine samples may be stored up to 36 hours at 2-8°C prior to assay. Specimens held for a longer time should be frozen only once at -20°C.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X Wash buffer into distilled water to yield 1X Wash buffer. The diluted wash buffer is stable for 2 weeks at RT.
- Standard 0-5: Reconstitute Standards with 0.75ml distilled water. The reconstituted standards are stable for 3 days at 2-8 °C or stored at -20 °C for longer period. See exact concentrations on the vial label.
- Sample dilution: If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with samples diluent and re-assay. For urine samples, dilute samples 1:20 with samples diluent.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (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. Add 100 µl of standards and samples in duplicate into wells.
3. Add 50 µl antiserum into all wells.
4. Add 100 µl C-peptide biotin into all wells.
5. Cover wells and incubate for 1 hours at RT with shaking (400-500 rpm).
6. Aspirate each well and wash, repeating the process 4 times for a total 5

washes. Wash by filling each well with 1× Wash Buffer (350 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.

7. Add 100 µl HRP conjugate solution into each well. Incubate for 30 mins at RT with shaking (400-500 rpm).
8. Wash as according to step 6.
9. Add 100 µl of TMB Reagent to each well. Incubate for 20 minutes at room temperature 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,

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.

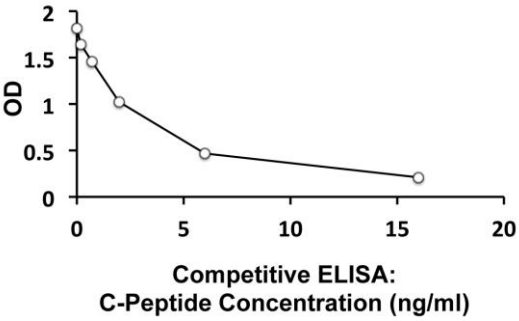
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

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.

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 total C-peptide ranged from 0.2-16 ng/ml. The mean MDD was 0.064 ng/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.12% and inter-assay precision was 9.2%.

Recovery

88.1-107.3% (serum samples); 96.2-109.2% (urine samples)

Linearity

97.1-112.8% (serum samples); 92.4-102.2% (urine samples)