Mouse C Peptide ELISA Kit

Enzyme Immunoassay for the quantitative determination of Mouse C Peptide in serum and plasma.

Catalog number: ARG82577

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

The connecting peptide, or C-peptide, is a short 31-amino-acid polypeptide that connects insulin's A-chain to its B-chain in the proinsulin molecule. In the context of diabetes or hypoglycemia, a measurement of C-peptide blood serum levels can be used to distinguish between different conditions with similar clinical features.

In the insulin synthesis pathway, first preproinsulin is translocated into the endoplasmic reticulum of beta cells of the pancreas with an A-chain, a C-peptide, a B-chain, and a signal sequence. The signal sequence is cleaved from the N-terminus of the peptide by a signal peptidase, leaving proinsulin. After proinsulin is packaged into vesicles in the Golgi apparatus (beta-granules), the C-peptide is removed, leaving the A-chain and B-chain bound together by disulfide bonds, which constitute the insulin molecule.

Patients with diabetes may have their C-peptide levels measured as a means of distinguishing type 1 diabetes from type 2 diabetes or Maturity onset diabetes of the young (MODY). Measuring C-peptide can help to determine how much of their own natural insulin a person is producing as C-peptide is secreted in equimolar amounts to insulin. C-peptide levels are measured instead of insulin levels because C-peptide can assess a person's own insulin secretion even if they receive insulin injections, and because the liver metabolizes a large and variable amount of insulin secreted into the portal vein but does not metabolise C-peptide, meaning blood C-peptide may be a better measure of portal insulin secretion than insulin itself.

Differential diagnosis of hypoglycemia. The test may be used to help determine the cause of hypoglycaemia (low glucose), values will be low if a

person has taken an overdose of insulin but not suppressed if hypoglycaemia is due to an insulinoma or sulphonylureas.

Factitious (or factitial) hypoglycemia may occur secondary to the surreptitious use of insulin. Measuring C-peptide levels will help differentiate a healthy patient from a diabetic one.

C-peptide may be used for determining the possibility of gastrinomas associated with Multiple Endocrine Neoplasm syndromes (MEN 1). Since a significant number of gastrinomas are associated with MEN involving other hormone producing organs (pancreas, parathyroids, and pituitary), higher levels of C-peptide together with the presence of a gastrinoma suggest that organs besides the stomach may harbor neoplasms.

C-peptide levels may be checked in women with Polycystic Ovarian Syndrome (PCOS) to help determine degree of insulin resistance.

[provided by Wikipedia: C-peptide]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for C Peptide has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any C Peptide present is bound by the immobilized antibody. After washing away any unbound substances, a HRP (Horseradish Peroxidase)-conjugated antibody specific for C Peptide is added to each well and incubate. 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 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 450nm ±2nm.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 and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C). Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	2 x 6 strips x 8-well	4°C
Standards	2 X 2.56 ng/vial	4°C
Sample/Standard Diluent	30 ml (ready-to-use)	4°C
HRP-anti-C Peptide Conjugate	13 ml (ready-to-use)	4°C
20X Wash Buffer	50 ml	4°C
TMB substrate	13 ml (ready-to-use)	4°C (Protect from light)
STOP solution	13 ml (ready-to-use)	4°C
Plate sealer	3 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 630 nm as reference wavelength)
- Pipettes and pipette tips
- Deionized or distilled water
- Vortex mixer
- 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 2-8°C at all times.
- Once Sample/Standard Diluent and TMB substrate is opened, the reagent is stable for one week at 2-8°C.
- Do not mix reagents that have different lot numbers.
- Briefly spin down the reconstituted **S**tandard before use.
- If crystals are observed in the 20X Wash buffer, warm to RT until the crystals are completely dissolved.
- Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 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.
- The wash procedure should be done thoroughly in order to minimize background readings.
- 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.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards and samples be assayed in duplicates.
- The same sequence of pipetting and other operations should be

maintained in all procedures.

- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

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 before centrifugation. Centrifuge samples (2000 x g) for 20 minutes at 2-8 °C. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma on ice using heparin (final concentration at 1 unit/ml), EDTA (final concentration at 0.1%), or sodium citrate (final concentration at 0.76%) as an anticoagulant. Centrifuge (2000 x g) for 20 minutes at 2-8 °C within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Avoid using hemolysis samples. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged to produce a clear solution.

REAGENT PREPARATION

- Antibody-coated microplate: Provided as ready to use. Remove the microplate from the foil pouch after the pouch has been equilibrated to room temperature.

  Note: The microplate must be used the same day as the pouch is opened.

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash Solution is stable for one week at 2-8℃. Prepare only enough as needed.
- Sample: If in an initial assay, a specimen is found to contain C Peptide concentration more than the highest standard, the specimens can be diluted with Sample/Standard Diluent and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.
- Standards: Reconstitute the standard with 200 µl Sample/Standard diluent buffer to yield a stock concentration of 12.8 ng/ml. Allow the stock standard to sit for few minutes with gentle invert the vail to make sure the standard is dissolved completely before making serial dilutions. The Sample/Standard diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 6.4 ng/ml, 3.2 ng/ml, 1.6 ng/ml, 0.8 ng/ml, 0.4 ng/ml, 0.2 ng/ml, 0.1 ng/ml.

Dilution table for Mouse C Peptide standard preparation:

C Peptide Concentration (ng/ml)	Volume of Sample/Standard diluent (µl)	Volume of standard (µl)
6.4	100	100 (12.8 ng/ml stock)
3.2	100	100 (6.4 ng/ml)
1.6	100	100 (3.2 ng/ml)
0.8	100	100 (1.6 ng/ml)
0.4	100	100 (0.8 ng/ml)
0.2	100	100 (0.4 ng/ml)
0.1	100	100 (0.2 ng/ml)
0	100	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use (for at least 30 minutes is needed) and should be stored at 2-8°C immediately after use. Before use, mix the reagents and samples thoroughly by gentle agitation or swirling. 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 **95 µl** of **Sample/Standard diluent** in each well.
3. Add **5 µl** of **standards and samples** in duplicate into the appropriate wells.
4. Cover the microplate with the plastic microplate cover and mix the solution in each well for 10 seconds (shake the microplate on level table

with hand or with microplate shaker).

5. Incubate the plate for **1 hours at RT**.
4. Aspirate each well and wash, repeating the process 5 times for a **total 6 washes.** Wash by filling each well with **1X Wash Buffer (300 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.
5. Add **100 µl** of **HRP-anti-C Peptide Conjugate** solution into each well. Mix thoroughly for 10 seconds. Cover the microplate and then incubate for **1 hour at RT.**
6. Aspirate each well and **wash as step 4**.
7. Immediately add **100 µl** of **TMB substrate** to each well. Incubate for **30 minutes at room temperature in dark**. (Do not cover the plate with aluminum foil.)
8. Add **100 µl** of **Stop Solution** to each well.
9. **Read** the OD with a microplate reader **at 450 nm** immediately. It is recommended that the wells be read within 30 minutes after adding the Stop Solution. (Optional: read at 630 nm as reference wavelength)

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

Note: If individual absorbance values differ from the mean by greater than 20%, performing the assay again is recommended.

2. Using semi-log, log-log or 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. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

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)

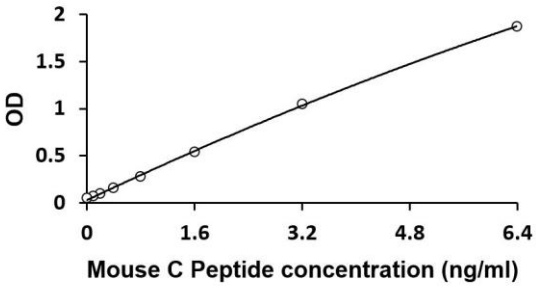
6. The mean absorbance of the 0 ng/ml standard should be less than 0.1.

7. The c-peptide concentration is expressed in ng/mL. The unit of measure can be converted to pM: 1 ng/ml = 320.3 pM.

8. If in an initial assay, a specimen is found to contain C Peptide concentration more than the highest standard (6.4 ng/ml), the specimens should be diluted with Sample/Standard Diluent and re-assayed as described in Assay Procedure. For the calculation of the concentrations this dilution factor has to be taken into account.

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.



The mean O.D. of the zero standard (0 ng/ml) should be <0.1.

QUALITY ASSURANCE

Sensitivity

Minimum Detectable Concentration:

0.1 ng/ml

Specificity

Substance	Cross-reactivity (%)
Mouse C-Peptide I	110
Mouse C-Peptide II	100
Rat C-Pepitide I	85
Rat C-Pepitide II	115
Mouse insulin	Not detected

Intra-assay and inter-assay precision

The CV value of intra-assay and inter-assay precisions were ≤ 10%

Recovery

80 - 120%