ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse/Rat C-peptide ELISA Kit

(Catalog Number: 36780)

For the quantitative determination of C-peptide in mouse/rat serum or plasma

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INTRODUCTION

Insulin is a kind of peptide hormone which is critical for glucose homeostasis. The mature Insulin peptide is derived from Proinsulin, which includes the Insulin A and B chains connected by a peptide fragment (C-peptide). Proinsulin is processed within the endoplasmic reticulum of pancreatic beta cells into equimolar ratios of mature Insulin and C-peptide. Mouse C-peptide 1 is a single chain peptide composed of 29 amino acids, while C-peptide 2 is composed of 31 residues. C-peptide is secreted together with insulin.The role of C-peptide has been considered to keep the best configuration to form three disulfide bonds, and has no biological activity, however, recent studies indicated that C-peptide can bind, probably, a G-protein coupling specific receptor present on the surface of endothelial cells, kidney microtubule cells and fibroblasts, resulting in activation of calcium-dependent intracellular signaling, activation of Na+-K+-ATPase, and enhancement of NO synthesis. Administration of C-peptide to DM1 patients enhances blood circulation in the skeletal muscle and skin, and also minimizes kidney glomerular hyperfiltration, decreasing albumin excretion into urine, and also improves nervous function, indicating that C-peptide should be given together with insulin to DM1 patients. Important region to bind receptor has been reported to be C-terminal penta peptide.

PRINCIPLE OF THE ASSAY

This assay is a two-site enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a monoclonal antibody against C-peptide. Standards and samples are added into the wells and co-incubated with a biotin labeled monoclonal antibody. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate is added and color develops in proportion to the amount of C-peptide bound initially. The assay is stopped, and the optical density of the wells is determined using a micro-plate reader. Since the increases in absorbance are directly proportional to the amount of captured C-peptide, the unknown sample concentration can be interpolated from a reference curve included in each assay.

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 C-peptide, sealed
2. 10×Wash buffer, 40 mL
3. Assay buffer, 30 mL, ready for use
4. 100×Detection antibody solution, a biotin labeled monoclonal antibody against C-peptide, 0.12 mL
5. C-peptide standard solutions, 8 ng/mL (0.15 mL), ready for use
6. 200×STP-HRP solution, 0.06 mL

7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use
9. 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
7. Horizontal micro-plate shaker capable of 600 rpm

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the 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×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 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.

B. 1×Detection antibody solution

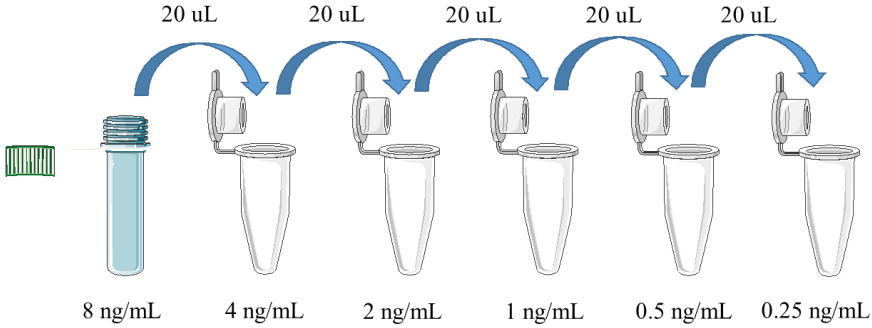
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C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

C. 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 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 AND SAMPLES

Mouse/Rat c-peptide Standards: Prepare serially diluted standards using 1×Assay buffer as shown below. 1×Assay buffer serves as the zero standard (0 pg/mL).



SAMPLE HANDLING

Serum or plasma sample generally requires a **2-fold** dilution in the 1×assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

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

1. Add 5 µL of standard or sample to its respective well.
2. Add 100 µL of 1xDetection antibody solution per well.
3. Seal the plate with a plate cover. Incubate at room temperature for 120 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
4. 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. Incubate at room temperature 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.
5. Add 100 µL of 1×STP-HRP solution to each well, seal the plate with a plate cover. Incubate at room temperature for 30 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.

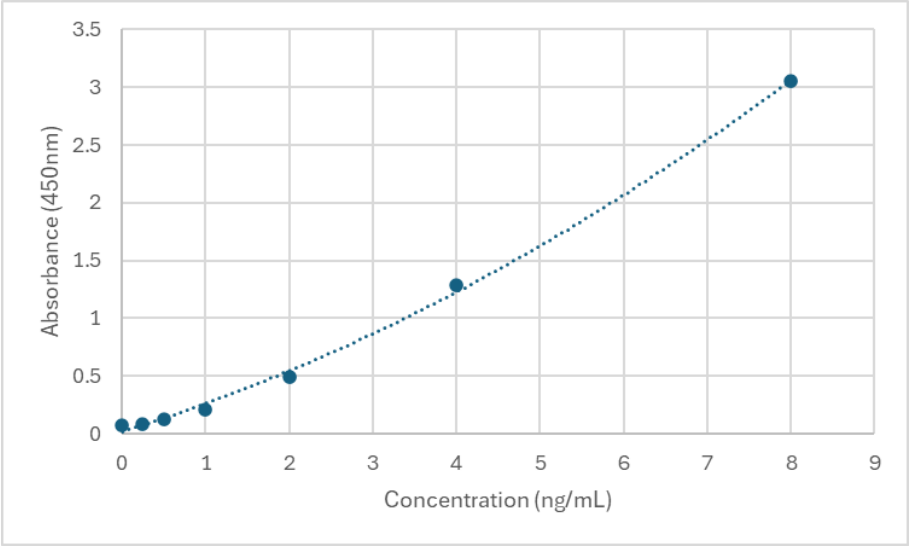
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against C-peptide concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Log-log curve fitting or curve of 4-parameter can be used for calculation.
3. Determine C-peptide 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 assay.

C-peptide (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.075	0
0.25	0.085	0.01
0.5	0.127	0.052
1	0.214	0.139
2	0.495	0.420
4	1.289	1.214
8	3.053	2.978

C-peptide standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest C-peptide level that can be measured by this assay is 0.14 ng/mL.

B. Precision

Intra-assay Precision (Precision within an assay) C.V. <5%.
Inter-assay Precision (Precision between assays) C.V. <10%.

SUMMARY OF ASSAY PROCEDURE

Add 5 µL of standard or sample to each well.
↓
Add 100 µL of 1×Detection antibody solution per well.
↓
Incubate at room temperature for 120 minutes (600 rpm).
↓
Wash each well 3 times.
↓
Add 100 µL of 1×STP-HRP solution to each well.
↓
Incubate at room temperature for 30 minutes (600 rpm).
↓
Wash each well 4 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