KAMIYA BIOMEDICAL COMPANY

Rat C-Peptide EIA

For the quantitative determination of C-Peptide in rat plasma, serum and urine.

Cat. No. KT-366

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Rat C-Peptide EIA is for the quantitative determination of C-Peptide in rat plasma, serum or urine. For research use only.

INTRODUCTION
This enzyme immunoassay (EIA) kit is a stable and convenient assay system for rat C-peptide in plasma, serum and urine. The processing of proinsulin, which occurs within the B cell, yields insulin and C-peptide and is secreted in equi-molar quantities into the blood circulation. Therefore, the measurement of C-peptide in blood reflects the concentration of insulin and also provides valuable information to evaluate the pancreatic B cell function. The EIA kit uses synthetic rat C-Peptide I as the calibrator and biotinylated rat C-Peptide I as the labeled antigen. The kit contains a specific polyclonal antibody that recognizes the amino acid sequence in the C-terminal region, which are common between rat C-Peptide I and II. The EIA kit has high specificity to rat C-Peptide and shows no cross reactivity to human and other animal species. The advantages of this assay include good quantification, no influence with other body fluid factors or physiological active substances and no sample pretreatment needed.

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a highly specific antibody to rat C-Peptide and a biotin/avidin-affinity system. The rat C-Peptide Calibrator is a highly purified synthetic product (purity: >98%) and the biotinylated peptide is stable to the N –biotinylglycylglycyl Rat C-Peptide I that is used. The 96-well plate is coated with goat anti-rabbit IgG and C-Peptide calibrator or samples, then biotinylated rat C-Peptide and anti-rat C-Peptide antibody are added to the wells for a competitive immuno-reaction. After rinsing excess rat C-Peptide, HRP-labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP-labeled streptoavidin - biotinylated rat C-Peptide – antibody complexes are formed on the surface on the wells. Finally, excess HRP-labeled streptoavidins are rinsed out, HRP-enzyme activity is determined, and the concentration of rat C-Peptide is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Anti-rabbit IgG
2. C-Peptide Calibrator	Lyophilized	1 vial (50 ng)	Synthetic rat C-Peptide I
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated rat C-Peptide I
4. C-Peptide Antibody	Liquid	1 bottle (12 mL)	Rabbit anti-rat C-Peptide
5. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
6. Substrate Buffer	Liquid	1 bottle (24 mL)	0.015% Hydrogen Peroxide
7. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine hydrochloride
8. Stop Solution	Liquid	1 bottle (12 mL)	2 N H_2SO_4
9. Buffer Solution	Liquid	1 bottle (35 mL)	Phosphate buffer
10. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
11. Plate Seal		3 sheets	

MTP[*1]..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate with aspiration system and dispenser for approximately 0.3 mL
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Test tubes for preparation of Calibrator Solution
- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample to avoid cross-contamination.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the C-Peptide Calibrator (lyophilized rat C-Peptide I, 50 ng/vial) with 1 mL of distilled water, giving a 50 ng/mL Calibrator Solution after reconstitution. 0.5 mL of the reconstituted Calibrator Solution is diluted with 0.5 mL of Buffer Solution to yield a 25 ng/mL Calibrator Solution. 0.5 mL of the 25 ng/mL Calibrator Solution is diluted with 0.5 mL of the Buffer Solution to make a 12.5 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 6.25, 3.12, and 1.56 ng/mL. Buffer Solution is used as the zero calibrator (0 ng/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

2. Preparation of Labeled Antigen: Reconstitute Labeled Antigen with 8 mL of distilled water.

   Note: Labeled Antigen must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Substrate Solution: Dissolve OPD Tablet in 11 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

4. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Diluted Wash Solution is stable for up to 6 months when stored at 4°C.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

5. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

SPECIMEN COLLECTION AND HANDLING

Plasma samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below –30°C. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature before beginning the test.
2. Fill 50 µL of Buffer Solution into wells first, then add 50 µL each of the prepared Calibrator Solutions (0, 1.56, 3.12, 6.25, 12.5, 25, 50 ng/mL) or samples. Then add 50 µL of Labeled Antigen and add 100 µL of C-Peptide Antibody to the wells.
3. Cover the plate with the Plate Seal and incubate at room temperature (20 - 30℃) for 3 hours. During the incubation, the plate should be rotated on a plate rotator.
4. Take off the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.35 mL/well of Wash Solution.
5. Pipette 100 µL of SA-HRP Solution into each of the wells.
6. Cover the plate with a Plate Seal and incubate at room temperature (20 - 30℃) for 2 hours. During the incubation, the plate should be rotated on a plate rotator.
7. Remove the Plate Seal, aspirate and wash the wells three times with approximately 0.35 mL/well of Wash Solution.
8. Add 100 µL of Substrate Solution into the wells, cover the plate with a Plate Seal and incubate for 10 minutes at room temperature.
9. Add 100 µL of Stop Solution into the wells to stop the reaction.
10. Read the optical absorbance of the wells at 490 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place polystyrene foam or plywood between the plate and the rotator. Test all samples in duplicate.

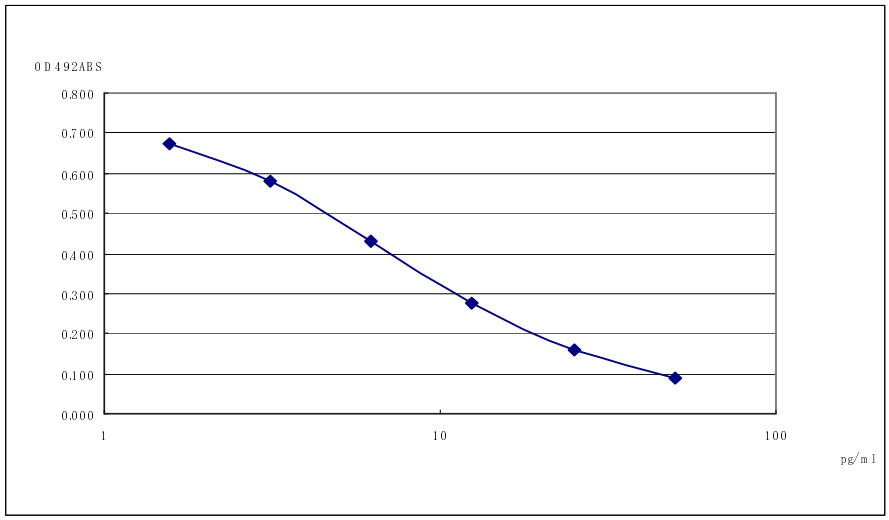
RESULTS

Calculate mean absorbance values of wells containing the Calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of Calibrators; ordinate: absorbance values of Calibrators). Use the calibration curve to read C-Peptide concentrations in samples from the corresponding absorbance values.

When a sample value exceeds 50 ng/mL, it must be diluted with Buffer Solution and re-assayed until the sample value is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

Rat C-Peptide Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	5.7	N/A	N/A
1.0	6.26	6.13	102.2%
5.0	10.2	10.1	100.6%
25.0	32.2	30.1	106.9%

Precision and reproducibility

- Intra-assay CV (%) 3.38 - 8.83
- Inter-assay CV (%) 5.56 - 8.41

Assay Range

1.56 – 50 ng/mL

FOR RESEARCH USE ONLY

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