Version 02-05/25

User's Manual

C-Peptide (U-type) Mouse ELISA Kit

REF **DEIA4507**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This is an ELISA (Enzyme Linked ImmunoSorbent Assay) kit for measurement of mouse C-peptide with high sensitivity using Sandwich assay principle.

General Description

C-peptide is formed from pro-insulin and co-secreted with insulin. Measuring the amount of c-peptide is useful as an index of insulin secretion. The Mouse C-Peptide ELISA kit is a simple, precise, and sensitive ELISA sandwich assay for mouse c-peptide.

Principles of Testing

The Mouse C-Peptide ELISA kit is an ELISA sandwich assay for mouse c-peptide. Mouse c-peptide in the sample is first bound to the rabbit anti-c-peptide antibody coated on the microplate well. POD conjugated anti-c-peptide antibody is then bound to the complex immobilized on the microplate. After addition of the substrate solution, the concentration of mouse c-peptide is interpolated from a standard curve based on the absorbance measured.

Reagents And Materials Provided

Mark	Description	Amount
A	Antibody-coated Microplate (One pack contains 6 x 8 wells, ie. 48 wells)	2 packs
B	Mouse C-Peptide Standard, Lyophilized	2 vials (2.56 ng/vial)
C	Anti-C-Peptide Enzyme Conjugate Solution	1 bottle (13 mL)
D	Enzyme Substrate (TMB) Solution	1 bottle (13 mL)
E	Enzyme Reaction Stop Solution (1 N Sulfuric Acid)	1 bottle (13 mL)
F	Sample Diluent	1 bottle (30 mL)
G	Wash Buffer Stock Solution (20X Concentrate)	1 bottle (50 mL)
	Frame for affixing the microplate well module	1 piece
	Plastic microplate cover	1 piece

Materials Required But Not Supplied

1. Micropipettes and disposable tips
2. Volumetric flasks
3. Distilled or deionized water
4. Polypropylene microtubes

5. Test tube racks
6. Vortex mixer
7. Aspirator for washing procedure
8. Microplate shaker (optional)
9. Microplate reader (capable of reading A450 and A630 values)

Storage

2-8°C, in a dark place. Do not freeze. Valid period is 6 months afterpreparation. Expiration date is shown on the label of the container.

Specimen Collection And Preparation

Plasma: Collect blood into a tube containing an anticoagulant such as heparin (final concentration: 1 unit/mL), EDTA (final concentration: 0.1%), or sodium citrate (final concentration: 0.76%), and centrifuge at 4°C for 20 min at 2,000 x g.

Serum: Collect blood, allow to clot, and centrifuge at 4°C for 20 min at 2,000 x g.

Please note to avoid hemolysis during preparation. Do not use turbid serum or plasma samples. Turbid serum or plasma should be centrifuged to produce a clear solution. Samples which need to be diluted must be diluted with the Sample Diluent.

Reagent Preparation

Preparation of reagents

1. 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.

2. Mouse c-peptide stock solution

Reconstitute the "Mouse C-Peptide Standard, Lyophilized" (marked "B") by careful addition of 200 µL of Sample Diluent (marked "F") to the vial. Invert the vial gently until the contents are completely dissolved. This stock solution contains 12.8 ng/mL of mouse c-peptide.

3. Anti-c-peptide enzyme conjugate

Provided as ready to use.

4. Enzyme substrate solution

Provided as ready to use. Once the bottle is opened, the enzyme substrate solution is stable for one week at 2-8°C.

Note: Avoid exposure of the enzyme substrate solution to light.

5. Enzyme reaction stop solution (1 N sulfuric acid)

Provided as ready to use.

6. Sample Diluent

Provided as ready to use. Once the bottle is opened, the sample diluent is stable for one week at 2-8°C.

7. Wash Buffer (20X Concentrated)

The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for one week at 2-8°C after dilution.

Preparation of working standards

1. Pipette 100 µL of Sample Diluent and 100 µL of the reconstituted standard (12.8 ng/mL) into a polypropylene microtube labeled 6.4 ng/mL, and mix thoroughly.
2. Dispense 100 µL of Sample Diluent into six polypropylene microtubes labeled 3.2, 1.6, 0.8, 0.4, 0.2, and 0.1 ng/mL,
3. Dispense 100 µL of the 6.4 ng/mL standard into the 3.2 ng/mL microtube, and mix thoroughly.
4. Dispense 100 µL of the 3.2 ng/mL standard into the 1.6 ng/mL microtube, and mix thoroughly.
5. Dispense 100 µL of the 1.6 ng/mL standard into the 0.8 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 100 µL of Sample Diluent into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 6.4, 3.2, 1.6, 0.8, 0.4, 0.2, 0.1, and 0 ng/mL.

TABLE 2 Preparation of working mouse c-peptide standards

	Mouse c-peptide concentration (ng/mL)							
	6.4	3.2	1.6	0.8	0.4	0.2	0.1	0
MCSS*(µL)	100							
SD**(µL)	100	100	100	100	100	100	100	100
		100	100	100	100	100	100	
Total (µL)	200	200	200	200	200	200	200	100

MCSS*: Mouse c-peptide stock solution (12.8 ng/mL)
SD**: Sample Diluent

Assay Procedure

Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Remove the antibody-coated microplate modules from the sealed foil pouch after the pouch has been equilibrated to room temperature. Affix the microplates to the supporting frame.
2. In each well, add 95 µL of Sample Diluent.
3. In each well, add 5 µL of sample or working standards.

Note: Each sample and working standard should be assayed in duplicate.

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 micropalte shaker).
5. Incubate the plate for 1 hour at room temperature.

6. Aspirate well contents and wash six times using 300 µL of Wash Buffer per well.

After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.

7. Add 1 00 µL of Anti C-Peptide Enzyme Conjugate in each well.
8. Cover the microplate with the plastic cover and incubate the plate for 1 hour at room temperature.
9. Aspirate well contents and wash six times using 300 µL of Wash Buffer per well.

After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.

10. Immediately dispense 100 µL per well of Enzyme Substrate Solution and react for 30 minutes at room temperature. During the enzyme reaction, avoid exposing the microplate to light.

Note: Do not cover the plate with aluminum foil.

11. Stop the reaction by adding 100 µL of Enzyme Reaction Stop Solution.
12. Measure absorbance within 30 minutes using a plate reader (measure A450 values and subtract A630 values).

Determining the c-peptide concentration

1. Determine the mean absorbance for each set of duplicate standards or samples.

Note: If individual absorbance values differ from the mean by greater than 20%, performing the assay again is recommended. The mean absorbance of the 0 ng/mL standard should be less than 0.1.

2. Using linear graph paper, construct the C-peptide standard curve by plotting the mean absorbance value for each standard on the Y axis versus the corresponding standard mouse C-peptide concentration on the X axis.

Figure 1 is an example of a typical standard curve generated by the ELISA assay.

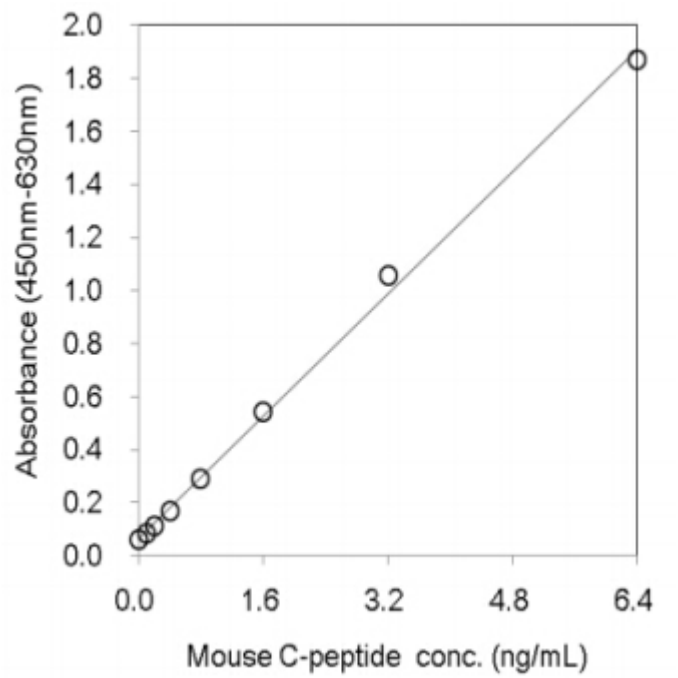
Note: A standard curve should be plotted every time the assay is performed.

3. Mouse C-peptide concentrations in the samples are interpolated using the standard curve and mean absorbance values for each sample. The c-peptide concentration is expressed in ng/mL. The unit of measure can be converted to pM by multiplying the obtained concentration in ng/mL by 320.3.

Note: Samples with high mouse c-peptide concentrations (ie. fall above 6.4 ng/mL) should be further diluted with the sample diluent and rerun.

Typical Standard Curve

FIGURE 1 A typical standard curve (linear fit)



Performance Characteristics

Maximizing Kit Performance

1. 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.
2. 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.
3. The wash procedure should be done thoroughly in order to minimize background readings.
4. Each calibrator and sample should be assayed in duplicate.
5. The same sequence of pipetting and other operations should be maintained in all procedures.
6. Do not mix reagents that have different lot numbers.

Precision

1. Within assay variation (2 samples, 8 fold assay) Average C.V. is 2.28%
2. Reproducibility (3 samples, triplicates assay, 4 days) C.V. is 0.07 ~ 3.46%

Detection Range

Assay range of the standard curve is 30-3000 pg/mL
According to the standard procedure where the samples are diluted 5 times, practical assay range is 150-15 000 pg/mL

Sensitivity

1.5 pg/mL

Recovery

When mouse c-peptide was spiked in a 5 µL mouse serum sample, the recovery was 100% ± 20%.

Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents, including the Enzyme Substrate Solution and the Enzyme Reaction Stop Solution, should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes. In case of contact with eyes or skin, flush immediately with water and contact a medical professional.
2. Do not allow the Enzyme Substrate Solution to contact any metal.
3. Do not use the reagents after the expiration date.