



Morinaga Mouse C-peptide ELISA Kit

For the quantitative determination of C-peptide in
mouse serum, plasma, and fluid

96wells

**For Laboratory Use Only, not for use in diagnostic procedure
Please read full descriptions in this manual before use.**

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A. *Intended Use*

The Morinaga Mouse C-peptide ELISA Kit is for the quantitative determination of C-peptide in mouse serum, plasma, and fluid. Please read the complete kit insert before performing this assay. The kit is for *Laboratory Use Only*. It is not intended for use in diagnostic procedures.

B. *Introduction*

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 Morinaga Mouse C-peptide ELISA Kit is a simple, precise, and sensitive ELISA sandwich assay for mouse C-peptide.

C. *Principles of the Assay*

1. First reaction

Mouse C-peptide in the sample is bound to the rabbit anti-C-peptide antibody coated on the microplate well.

2. Washing

Unbound material is removed by washing.

3. Second reaction

Horse radish peroxidase (POD)-conjugated anti-C-peptide antibody is then bound to the rabbit anti-C-peptide antibody/mouse C-peptide complex immobilized to the microplate well.

4. Washing

Excess POD-conjugate is removed by washing.

5. Enzyme reaction

The bound POD conjugate in the microplate well is detected by the addition of the 3, 3', 5, 5'-tetramethylbenzidine (TMB) substrate solution.

6. Measurement of absorbance

7. Evaluation of results

The C-peptide concentration is determined via interpolation using the standard curve generated by plotting absorbance versus the corresponding concentration of mouse C-peptide standard.

D. *Kit Storage*

1. Upon receipt of the Morinaga Mouse C-peptide ELISA Kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
A	Antibody-coated Microplate (One pack contains 6 x 8 well modules, i.e. 48 wells / pack)	2 packs
B	Mouse C-peptide Standard, Lyophilized	2 vials (2.56ng/vial)
C	Anti-C-peptide Enzyme Conjugate Solution	1 bottle (13 mL)
D	Enzyme Substrate (TMB) Solution	1 bottle (13 mL)
E	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
	Plate Sealers	3 sheets

E.2. Materials to be supplied by user

Micropipettes and disposable tips

Volumetric flasks

Distilled or deionized water

Polypropylene microtubes

Test tube racks

Vortex mixer

Aspirator for washing procedure

Microplate shaker (optional)

Microplate reader (capable of measuring absorbance at 450 nm and any wavelength between 610 to 650 nm, inclusive)

F. Assay 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 (marked "D") and the Stop Solution (marked "E"), 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 (marked "D") to contact any metal.
3. Do not use the reagents after the expiration date.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 μ L), pipetting should be done as carefully as possible. A high quality 10 μ L 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.

H. Sample Collection

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.

Note: 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 (marked "F").

I. Assay Procedure







I.1. 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 (20-30°C).
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 μ L 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 Solution
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. 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
The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. 50 mL of "Wash Buffer Stock Solution" (marked "G") must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for one week at 2-8°C after dilution.

I.2. 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 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

I.3. 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.

First reaction:

1. Remove the Antibody-coated Microplate (marked "A") from the sealed foil pouch after the pouch has been equilibrated to room temperature (20-30°C). Affix the microplates to the supporting frame.
2. In each well, add 95 μL of Sample Diluent (marked "F").
3. In each well, add 5 μL of sample or working standards.
Note: Each sample and working standards should be assayed in duplicate.
4. Cover the microplate with the plate sealer and mix the solution in each well for 10 seconds (shake the microplate on level table with hand or with micropalpe shaker).
5. Incubate the plate for 1 hour at room temperature (20-30°C).

Second reaction:

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 100 μ L of Anti C-peptide Enzyme Conjugate Solution (marked "C") in each well.
8. Cover the microplate with the plate sealer and incubate the plate for 1 hour at room temperature (20-30°C).

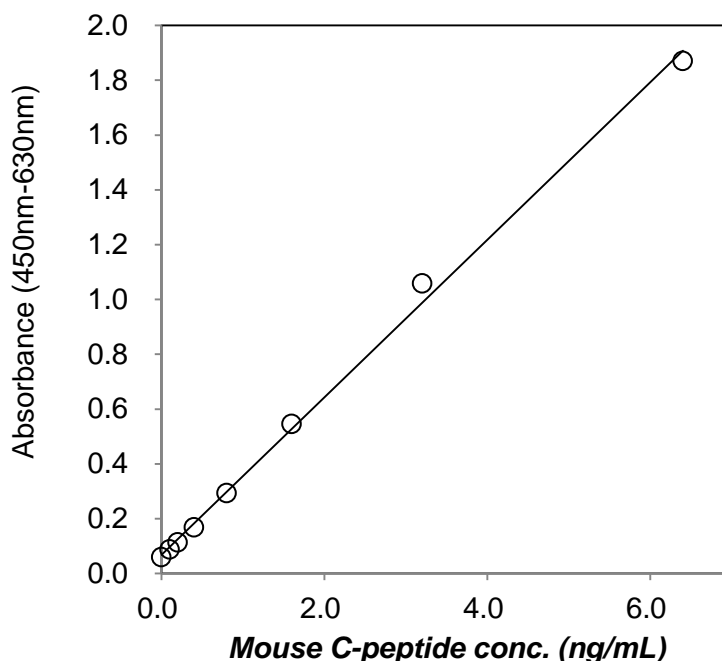
Third reaction:

9. Aspirate well contents and wash six times using 300 μ L 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 μ L per well of Enzyme Substrate Solution (marked "D")
11. Cover the microplate with the plate sealer and incubate the plate for 30 minutes at room temperature (20-30°C). During the enzyme reaction, avoid exposing the microplate to light.
Note: *Do not cover the plate with aluminum foil.*
12. Stop the reaction by adding 100 μ L of Stop Solution (marked "E").
13. Measure absorbance within 30 minutes using a plate reader (Measure absorbance at 450 nm, and subtract reference wave length: any wavelength between 610 to 650 nm, inclusive)

I.4. 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.*

FIGURE 1. A typical standard curve (linear fit)



J. Appendix

J.1. Performance characteristics

1. Assay range

The Morinaga Mouse C-peptide ELISA Kit has an assay range from 0.1 – 6.4 ng/mL.

2. Precision

The assay has a intra- and inter-assay precision of CV \leq 10%.

3. Recovery

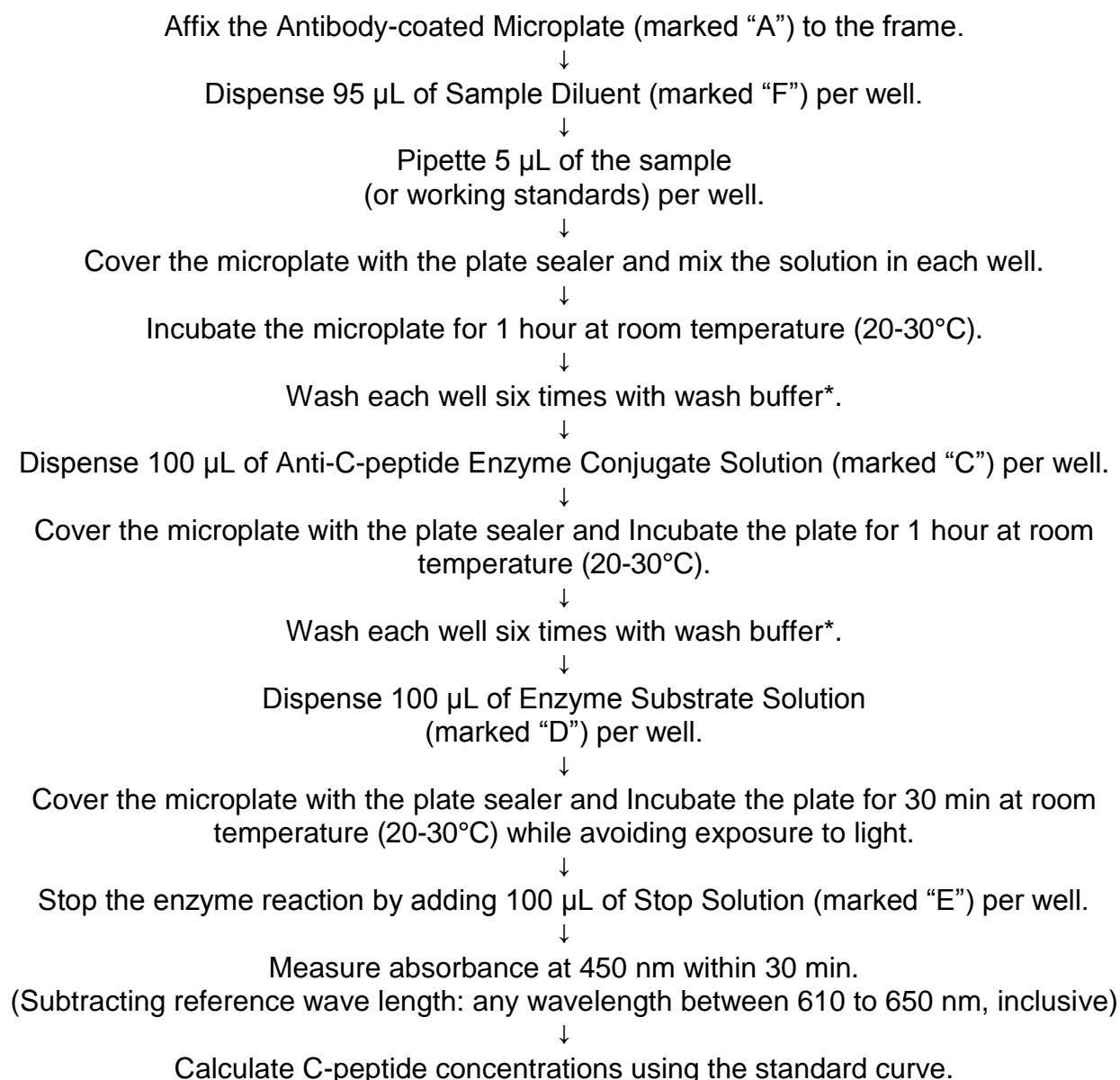
When mouse C-peptide was spiked in a 5 μ L mouse serum sample, the recovery was 100% \pm 20%.

J.2. Summary of reagent preparation

TABLE 3 Summary of reagent preparation

Reagent	Preparation Procedure
A: Antibody-coated Microplate	Ready to use
B: Mouse C-peptide Standard, Lyophilized	Dilute with 200 μ L of Sample Diluent
C: Anti-C-peptide Enzyme Conjugate Solution	Ready to use
D: Enzyme Substrate (TMB) Solution	Ready to use
E: Stop Solution (1N Sulfuric Acid)	Ready to use
F: Sample Diluent	Ready to use
G: Wash Buffer Stock Solution (20X Concentrate)	Bring contents of the bottle to 1L with distilled/deionized water

J.3. Summary of ELISA Assay



* Each well should be washed with 300 μ L of wash buffer. Aspirate the wells completely so all excess solution is removed.

Warranty

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