MSD® MULTI-SPOT Assay System

Mouse/Rat C-peptide Kit

1-Plate Kit	K150OID-1
5-Plate Kit	K150OID-2
25-Plate Kit	K150OID-4

MSD Metabolic Assays

Mouse/Rat C-peptide Kit

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

MESO SCALE DISCOVERY®
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Ordering Information

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Introduction

Connecting peptide, or C-peptide, is a 31 amino acid protein constituent of proinsulin produced by pancreatic β-cells in the Islets of Langerhans. C-peptide connects the A-chain and the B-chain of the proinsulin polypeptide and facilitates proper processing of proinsulin in the endoplasmic reticulum. Proinsulin is cleaved by Ca^{2+}-dependent endopeptidases; equimolar quantities of active insulin and C-peptide are stored in secretory granules, released into the portal vein, enter circulation, and are excreted by the kidneys.[1] Circulating C-peptide binds multiple cell types, including endothelial cells, fibroblasts, neuronal cells, and renal tubular cells. Bound C-peptide activates Ca^{2+}-dependent signaling pathways, including MAPK, PLCγ, and PKC, resulting in both increased eNOS and $Na^{+}K^{+}$ATPase activity and upregulation of transcription factors.[2]

Plasma levels of C-peptide have traditionally been used to distinguish between Type 1 and Type 2 diabetes.[3] Type 1 diabetics fail to produce proinsulin so that C-peptide levels are decreased, while Type II diabetics produce increased quantities of proinsulin so that C-peptide levels are increased. C-peptide is an active biomolecule of interest in the study of hormone-dependent conditions, including gastromas associated with multiple endocrine neoplasms syndrome, polycystic ovarian syndrome, metabolic syndrome X, and sub-sets of renal disease.[4]

Principle of the Assay

MSD metabolic assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Mouse/Rat C-peptide is a sandwich immunoassay (Figure 1). MSD provides a plate pre-coated with capture antibodies. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into a SECTOR® Imager where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample.

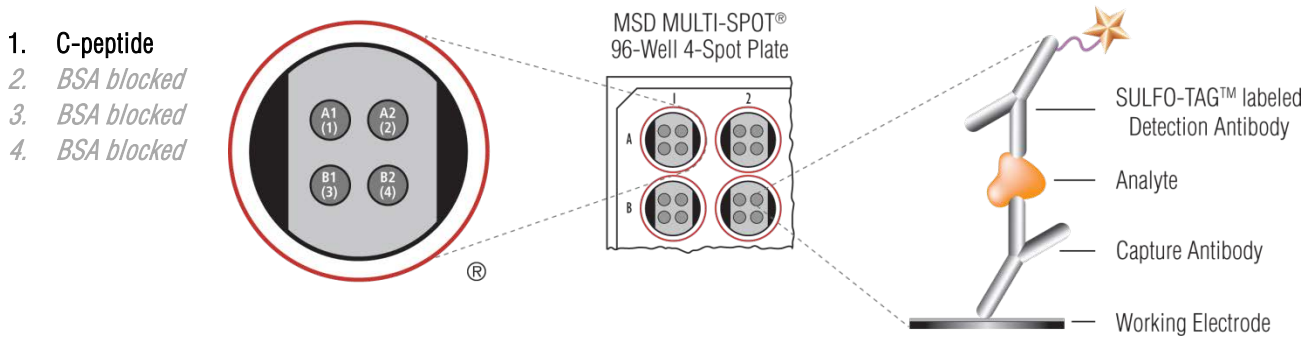


Figure 1. *Spot diagram showing placement of analyte capture antibodies. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files. A unique bar code label on each plate allows complete traceability back to MSD manufacturing records.*

Reagents Supplied

Product Description	Storage	Quantity per Kit K1500ID-1	K1500ID-2	K1500ID-4
MULTI-SPOT® 96-Well, 4-Spot Mouse/Rat C-peptide Plate N4500IA-1	2–8°C	1 plate	5 plates	25 plates
SULFO-TAG Anti-ms/rat C-peptide Antibody[1] (50X)	2–8°C	1 vial (75 µL)	1 vial (375 µL)	5 vials (375 µL ea)
Mouse/Rat C-peptide Calibrator (1 µg/mL)	≤-70°C	1 vial (60 µL)	5 vials (60 µL ea)	25 vials (60 µL ea)
Diluent 13 R56BB-4 (10 mL), R56BB-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL)	5 bottles (50 mL ea)
Diluent 12 R50JA-4 (10 mL), R50JA-3 (50 mL)	≤-10°C	1 bottle (10 mL)	1 bottle (50 mL ea)	5 bottles (50 mL ea)
Blocker A Kit (Blocker A [dry] in 250 mL bottle and 50 mL bottle of 5X Phosphate Buffer) R93AA-2 (250 mL)	RT	1 kit	1 kit	5 kits
Read Buffer T (4X) R92TC-3 (50 mL)	RT	1 bottle	1 bottle	5 bottles

Required Material and Equipment (not supplied)

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Microcentrifuge tubes for preparing serial dilutions
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 (PBS-T) for plate washing
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Adhesive plate seals
- ❑ Microtiter plate shaker
- ❑ Deionized water

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional safety information is available in the product Material Safety Data Sheet, which can be obtained from MSD Customer Service.

[1] SULFO-TAG–conjugated detection antibodies should be stored in the dark.

Reagent Preparation

Bring all reagents to room temperature. Thaw the stock calibrator on ice.

> **Important:** Upon first thaw, separate diluents 12 and 13 into aliquots appropriate for the size of your needs before refreezing.

Prepare Blocker A Solution

Follow the instructions included in the Blocker A kit.

Prepare Standards

MSD supplies calibrator for the Mouse/Rat C-peptide Kit at 20-fold higher concentration than the recommended highest standard. We recommend a 7-point standard curve with 3-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the standard curve solutions.

Standard	C-peptide (pg/mL)	Dilution Factor
Stock Calibrator	1 000 000	
STD-01	50 000	20
STD-02	16 667	3
STD-03	5556	3
STD-04	1857	3
STD-05	617	3
STD-06	206	3
STD-07	69	3
STD-08	0	n/a

To prepare 7 standard solutions plus a zero calibrator blank for up to 4 replicates:

1) Prepare the highest standard by adding 25 µL of stock calibrator to 475 µL of Diluent 13. Mix well.
2) Prepare the next standard by transferring 150 µL of the highest standard to 300 µL of Diluent 13. Mix well. Repeat 3-fold serial dilutions 5 additional times to generate 7 standards.
3) Use Diluent 13 as the blank.

Dilute Samples

For mouse or rat serum and plasma samples, MSD recommends assaying samples without dilution; however, you may need to incorporate a dilution for the sample set under investigation. Samples should be diluted in Diluent 13, if necessary.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working detection antibody solution is 1X.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-ms/rat C-peptide Antibody
- ❑ 2.94 mL of Diluent 12

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may prepare diluted read buffer in advance and store it at room temperature in a tightly sealed container.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates can be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

Notes

1. **Add Blocker A Solution:** Add 150 µL of Blocker A solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.

   Shaking the plate typically accelerates capture at the working electrode.

2. **Wash and Add Sample:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 50 µL of sample (standards, controls, or unknowns) per well. Seal the plate with an adhesive plate seal and incubate for 2 hours with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare detection antibody solution during incubation.

3. **Wash and Add Detection Antibody Solution:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Seal the plate with an adhesive plate seal and incubate for 1 hour with vigorous shaking (300–1000 rpm) at room temperature.
   You may prepare diluted read buffer during incubation.

   You may keep excess diluted read buffer in a tightly sealed container at room temperature for later use.

   Bubbles introduced when adding read buffer will interfere with imaging of the plate and produce unreliable data. Use reverse pipetting technique to avoid creating bubbles.

4. **Wash and Read:** Wash the plate 3 times with 150–300 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Analyze the plate on the SECTOR Imager. No incubation in read buffer is required before reading the plate.

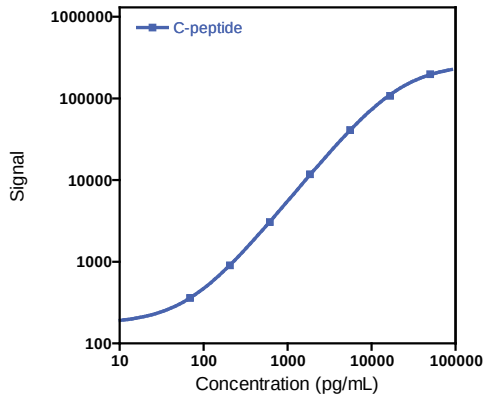
   Due to the varying nature of each research application, you should assess assay stability before allowing plates to sit with read buffer for extended periods.

Curve Fitting

MSD DISCOVERY WORKBENCH® software uses least-squares fitting algorithms to generate the standard curve that will be used to calculate the concentration of analyte in the samples. The assays have a wide dynamic range (3–4 logs) that allows accurate quantification without the need for dilution in many cases. By default, the software uses a 4-parameter logistic model (or sigmoidal dose-response) and includes a $1/Y^2$ weighting function. The weighting function is important because it provides a better fit of data over a wide dynamic range, particularly at the low end of the standard curve.

Typical Data

The following standard curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a standard curve for each plate using a minimum of 2 replicates of the standards.



C-peptide		
Conc. (pg/mL)	Average Signal	%CV
0	146	10.7
69	361	1.2
206	905	1.2
617	3 064	4.5
1 852	11 829	5.8
5 556	41 065	8.2
16 667	107 754	2.9
50 000	198 594	1.8

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration based on a signal 2.5 standard deviations above the background (zero calibrator blank). The LLOD shown below was calculated based on 3 runs.

	C-peptide
Average LLOD (pg/mL)	25
LLOD Range (pg/mL)	18–32

Tested Samples

Normal mouse plasma and rat serum samples were collected and tested with the Mouse/Rat C-peptide Kit. Median and range of concentrations for each sample set are displayed below.

Sample Type	Statistic	C-peptide
Mouse EDTA plasma	Median (pg/mL)	335
	Range (pg/mL)	284–391
	Number of Samples	4
	Samples in Quantitative Range	4
Rat serum	Median (pg/mL)	209
	Range (pg/mL)	108–693
	Number of Samples	3
	Samples in Quantitative Range	3

Assay Components

Calibrator

The assay calibrator uses synthetic C-peptide, residues 33-63, of the rat proinsulin molecule.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
C-peptide	Mouse Monoclonal	Mouse Monoclonal	A

References

1. Steiner DF, et al. Insulin biosynthesis: evidence for a precursor. Science. 1967 Aug 11;157(3789):697-700.
2. Hills CE, et al. Intracellular signaling by C-peptide. Exp Diabetes Res. 2008;2008:635158.
3. Vasic C, et al. C-peptide: a new mediator of artherosclerosis in diabetes. Mediators Inflamm. 2012;2012:858692.
4. Wahren J. C-peptide: new findings and therapeutic implications in diabetes. Clin Physiol Funct Imaging. 2004 Jul;24(4):180-9.

Summary Protocol

Mouse/Rat C-peptide Kit

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Mouse/Rat C-peptide assay.

Sample and Reagent Preparation

Bring all reagents to room temperature and thaw the calibrator on ice.

Prepare Blocker A solution.

Prepare standard solutions using the supplied calibrator:

- Dilute the stock calibrator 20-fold in Diluent 13.
- Perform a series of 3-fold dilution steps and prepare a zero calibrator blank.

Prepare detection antibody solution by diluting stock detection antibody 50-fold in Diluent 12.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Add Blocker A Solution

Add 150 µL/well of Blocker A solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 2: Wash and Add Sample

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 50 µL/well of sample (standards, controls, or unknowns).

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 2 hours.

Step 3: Wash and Add Detection Antibody Solution

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Incubate at room temperature with vigorous shaking (300–1000 rpm) for 1 hour.

Step 4: Wash and Read Plate

Wash plate 3 times with 150–300 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on SECTOR Imager.

Plate Diagram