

Human C-Peptide ELISA Kit

Enzyme-linked Immunosorbent Assay for quantitative detection of human C-Peptide

Catalog Number BMS2191-2

Pub. No. MAN0027986 Rev. B (31)



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Human C-Peptide ELISA Kit is an enzyme-linked immunosorbent assay for the quantitative detection of human C-Peptide. Cell culture supernatant, urine, serum, and plasma (EDTA, citrate) have been tested with this assay.

C-Peptide or also called connecting peptide is a polypeptide that connects insulin A and B chain. *In vivo* studies in animal models for diabetes 1 showed that administration of C-Peptide leads to improvement in nerve and kidney function. It has been observed that C-Peptide also has anti-inflammatory effects and plays an important role in emergency repair of smooth muscle cells.

Principles of the test

An anti-human C-Peptide coating antibody is adsorbed onto microwells.

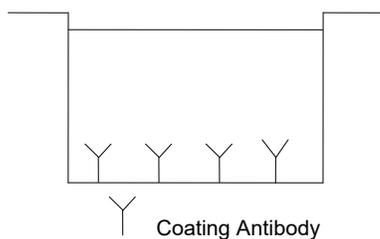


Figure 1 Coated microwell

Human C-Peptide present in the sample or standard binds to antibodies adsorbed to the microwells.

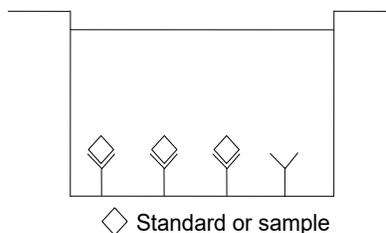


Figure 2 First incubation

A biotin-conjugated anti-human C-Peptide antibody is added and binds to C-Peptide captured by the first antibody.

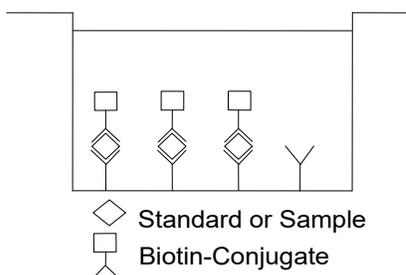


Figure 3 Second incubation

Following incubation, unbound biotin-conjugated anti-human C-Peptide antibody is removed during a wash step. Streptavidin-HRP is added and binds to the biotin-conjugated anti-human C-Peptide antibody.

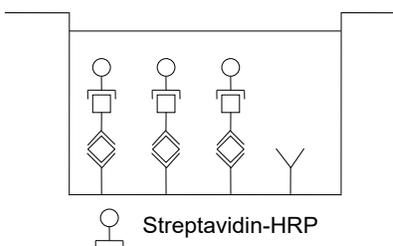


Figure 4 Third incubation

Following incubation, unbound Streptavidin-HRP is removed during the wash step, and substrate solution reactive with HRP is added to the wells.

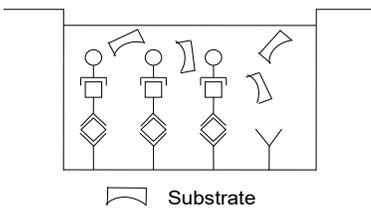


Figure 5 Fourth incubation

A coloured product is formed in proportion to the amount of C-Peptide present in the sample or standard. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from 7 C-Peptide standard dilutions and C-Peptide sample concentration determined.

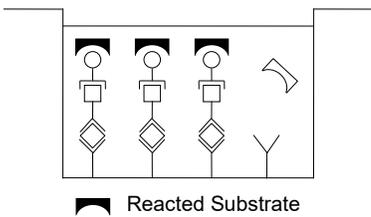


Figure 6 Stop reaction

Reagents provided

Reagents for human C-Peptide ELISA BMS2191-2 (96 tests)

- 1 aluminum pouch with a Microwell Plate coated with monoclonal antibody to human C-Peptide
- 1 vial (120 µL) Biotin-Conjugate anti-human C-Peptide monoclonal antibody
- 1 vial (150 µL) Streptavidin-HRP
- 2 vials human C-Peptide Standard lyophilized, 3000 pg/mL upon reconstitution
- 1 bottle (12 mL) Sample Diluent
- 1 vial (5 mL) Assay Buffer Concentrate 20x (PBS with 1% Tween™ 20, 10% BSA)
- 1 bottle (50 mL) Wash Buffer Concentrate 20x (PBS with 1% Tween™ 20)
- 1 vial (15 mL) Substrate Solution (tetramethyl-benzidine)
- 1 vial (15 mL) Stop Solution (1M Phosphoric acid)
- 6 Adhesive Films

Storage instructions – ELISA kit

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2° to 8°C). Expiry of the kit and reagents is stated on labels.

Expiry of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Sample collection and storage instructions

- Cell culture supernatant, serum, plasma (EDTA and citrate) and urine were tested with this assay. Other biological samples might be suitable for use in the assay.
- Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic samples.
- Samples should be aliquoted and must be stored frozen at –20°C to avoid loss of bioactive human C-Peptide. If samples are to be run within 24 hours, they may be stored at 2–8°C.
- Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Required materials not supplied

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single channel micropipettes with disposable tips
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microwell strip reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionized water
- Statistical calculator with program to perform regression analysis

Procedural guidelines

- Consider all reagents as potentially hazardous. Only trained personnel should handle this product, following good laboratory practice guidelines.
- Wear appropriate protective clothing, such as laboratory overalls, safety glasses, and gloves. Avoid contact with skin and eyes. If contact occurs, wash immediately with water. See the material safety data sheet (MSDS) or safety statement for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or replace reagents with those from other lots or other sources.
- Do not use kit reagents past the expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipet by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.

- Wear rubber or disposable latex gloves while handling kit reagents or samples.
- Avoid contact of the substrate solution with oxidizing agents and metal.
- Avoid splashing or generation of aerosols.
- Use disposable pipette tips and pipettes to prevent microbial contamination or cross-contamination of reagents and samples, which can invalidate the test.
- Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- Do not expose the HRP-Conjugate to an acid to prevent inactivation.
- Use glass-distilled water or deionized water for reagent preparation.
- Equilibrate the Substrate Solution to room temperature (18–25°C) before use.
- Decontaminate, then discard samples and potentially contaminated materials as if they contain infectious agents. The preferred method of decontamination is autoclaving for at least 1 hour at 121.5°C.
- Mix liquid waste that does not contain an acid and neutralized waste with sodium hypochlorite to obtain a final concentration of 1.0% sodium hypochlorite. Allow 30 minutes for successful decontamination. Neutralize liquid waste that contains an acid before the addition of sodium hypochlorite.
- Room temperature is defined as the temperature range 18–25°C.
- After the test begins, complete all steps without interruption.
- We recommend running calibrators, controls, and samples in duplicate (at a minimum).
- Follow the incubation times specified in this user guide.

Before you begin

- Equilibrate the buffer concentrates to room temperature, then dilute before use.
- If crystals have formed in the buffer concentrates, warm gently to dissolve the crystals.

Prepare reagents

Prepare Wash Buffer (1X)

1. Pour entire contents (50 mL) of the Wash Buffer Concentrate (20X) into a clean 1000 mL graduated cylinder. Bring to final volume of 1000 mL with glass-distilled or deionized water. Mix gently to avoid foaming.

2. Transfer to a clean wash bottle and store at 2–25°C.

Note: Wash Buffer (1X) is stable for 30 days.

3. Wash Buffer (1X) may also be prepared as needed according to the following table:

Number of Strips	Wash Buffer Concentrate (20X) (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Prepare Assay buffer (1X)

1. Pour the entire contents (5 mL) of the Assay Buffer Concentrate (20X) into a clean 100 mL graduated cylinder. Bring to final volume of 100 mL with distilled water. Mix gently to avoid foaming.

2. Store at 2–8°C.

Note: The Assay Buffer (1X) is stable for 30 days.

3. Assay Buffer (1X) may also be prepared as needed according to the following table:

Number of Strips	Assay Buffer Concentrate (20X) (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

Prepare Biotin-Conjugate

IMPORTANT! The Biotin-Conjugate should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1X) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1X) (mL)
1–6	0.06	5.94
1–12	0.12	11.88

Prepare Streptavidin-HRP

IMPORTANT! The Streptavidin-HRP should be used within 30 minutes after dilution.

Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1X) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1X) (mL)
1– 6	0.06	5.94
1–12	0.12	11.88

Prepare Human C-Peptide standard

1. Reconstitute human C-Peptide standard by addition of distilled water.
2. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 3000 pg/mL).
3. Allow the standard to reconstitute for 10–30 minutes. Mix well prior to making dilutions.
4. After usage remaining standard cannot be stored and has to be discarded.
5. Standard dilutions can be prepared directly on the microwell plate (see “Test protocol” on page 4) or alternatively in tubes (see “External standard dilution” on page 4).

External standard dilution

1. Label 7 tubes, one for each standard point: S1, S2, S3, S4, S5, S6, S7.
2. Prepare 1:2 serial dilutions for the standard curve as follows: Pipette 225 µL of Sample Diluent into each tube.
3. Pipette 225 µL of reconstituted standard (concentration = 3000 pg/mL) into the first tube, labeled S1, and mix (concentration of standard 1 = 1500 pg/mL).
4. Pipette 225 µL of this dilution into the second tube, labeled S2, and mix thoroughly before the next transfer.

5. Repeat serial dilutions 5 more times thus creating the points of the standard curve (see Figure 7).

Sample Diluent serves as blank.

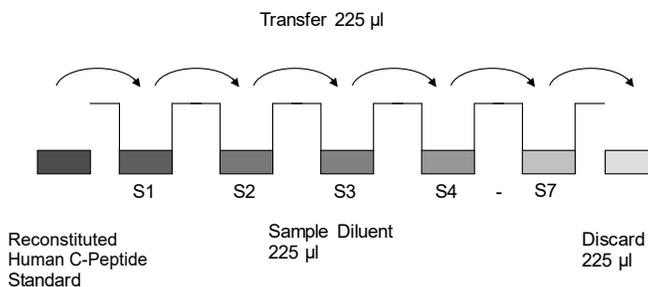


Figure 7 Dilute standards - tubes

Test protocol

IMPORTANT!

- Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards.
- Each sample, standard, blank and optional control sample should be assayed in duplicate.
- Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2–8°C sealed tightly.

1. Wash the microwell strips twice with approximately 400 µL Wash Buffer per well with thorough aspiration of microwell contents between washes. Allow the Wash Buffer to sit in the wells for about 10–15 seconds before aspiration.

Note: Do not scratch the surface of the microwells.

After the last wash step, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess Wash Buffer. Use the microwell strips immediately after washing. Alternatively microwell strips can be placed upside down on a wet absorbent paper for not longer than 15 minutes. Do not allow wells to dry.

2. Standard dilution on the microwell plate (Alternatively the standard dilution can be prepared in tubes - see “External standard dilution” on page 4)
 - a. Add 100 µL of Sample Diluent in duplicate to all standard wells.
 - b. Pipette 100 µL of prepared standard (see “Prepare Human C-Peptide standard” on page 4, concentration = 3000 pg/mL) in duplicate into well A1 and A2 (see Table 1).
 - c. Mix the contents of wells A1 and A2 by repeated aspiration and ejection (concentration of standard 1, S1 = 1500 pg/mL), and transfer 100 µL to wells B1 and B2, respectively (see Figure 8).

Note: Do not scratch the inner surface of the microwells.
 - d. Continue this procedure 5 times, creating two rows of human C-Peptide standard dilutions ranging from 1500 to 23 pg/mL.

- e. Discard 100 μ L of the contents from the last microwells (S7) used.

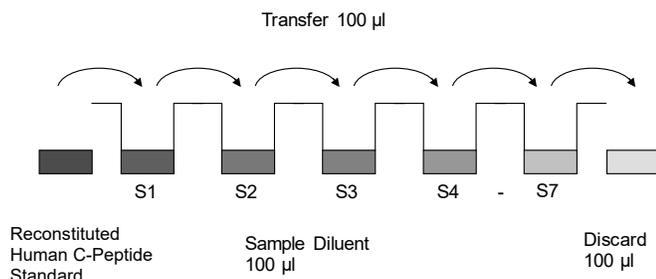


Figure 8 Dilute standards - microwell plate

Table 1 Table depicting an example of the arrangement of blanks, standards and samples in the microwell strips:

	1	2	3	4
A	Standard 1 (1500 pg/mL)	Standard 1 (1500 pg/mL)	Sample 1	Sample 1
B	Standard 2 (750 pg/mL)	Standard 2 (750 pg/mL)	Sample 2	Sample 2
C	Standard 3 (375 pg/mL)	Standard 3 (375 pg/mL)	Sample 3	Sample 3
D	Standard 4 (188 pg/mL)	Standard 4 (188 pg/mL)	Sample 4	Sample 4
E	Standard 5 (94 pg/mL)	Standard 5 (94 pg/mL)	Sample 5	Sample 5
F	Standard 6 (47 pg/mL)	Standard 6 (47 pg/mL)	Sample 6	Sample 6
G	Standard 7 (23 pg/mL)	Standard 7 (23 pg/mL)	Sample 7	Sample 7
H	Blank	Blank	Sample 8	Sample 8

In case of an external standard dilution (see “External standard dilution” on page 4), pipette 100 μ L of these standard dilutions (S1– S7) in the standard wells according to Table 1.

- Add 100 μ L of Sample Diluent in duplicate to the blank wells.
- Add 50 μ L of Sample Diluent to the sample wells.
- Add 50 μ L of each sample in duplicate to the sample wells.
- Cover with an adhesive film and incubate at room temperature for 2 hours, on a microplate shaker.
- Prepare Biotin-Conjugate (see “Prepare Biotin-Conjugate” on page 3).
- Remove adhesive film and empty wells. Wash microwell strips 6 times according to point step 1 of the test protocol.
- Add 100 μ L of Biotin-Conjugate to all wells, including blank wells.
- Cover with an adhesive film and incubate at room temperature for 1 hour, if available on a microplate shaker.
- Prepare Streptavidin-HRP (see Preparation of Streptavidin-HRP on page 4).

- Remove adhesive film and empty wells. Wash microwell strips 6 times according to point step 1 of the test protocol.
- Pipette 100 μ L of diluted Streptavidin-HRP to all wells, including blanks.
- Cover with an adhesive film and incubate at room temperature for 30 minutes on a microplate shaker.
- Remove adhesive film and empty wells. Wash microwell strips 6 times according to point step 1 of the test protocol.
- Pipette 100 μ L of TMB Substrate Solution to all wells.
- Incubate the microwell strips at room temperature for about 30 minutes. Avoid direct exposure to intense light.

The color development on the plate should be monitored and the substrate reaction stopped (see next point of this protocol) before positive wells are no longer properly recordable. Determination of the ideal time period for color development has to be done individually for each assay.

It is recommended to add the stop solution when the highest standard has developed a dark blue color. Alternatively the color development can be monitored by the ELISA reader at 620 nm. The substrate reaction should be stopped as soon as Standard 1 has reached an OD of 0.9–0.95.

- Stop the enzyme reaction by quickly pipetting 100 μ L of Stop Solution into each well. It is important that the Stop Solution is spread quickly and uniformly throughout the microwells to completely inactivate the enzyme. Results must be read immediately after the Stop Solution is added or within one hour if the microwell strips are stored at 2–8°C in the dark.
- Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer’s instructions by using the blank wells. Determine the absorbance of both the samples and the standards.

Note: If instructions of this protocol have been followed samples have been diluted 1:2, the concentration read from the standard curve must be multiplied by the dilution factor (x2).

Calculation of results

- Calculate the average absorbance values for each set of duplicate standards and samples. Duplicates should be within 20 percent of the mean value.
- Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the human C-Peptide concentration on the abscissa. Draw a best fit curve through the points of the graph (a 5-parameter curve fit is recommended).
- To determine the concentration of circulating human C-Peptide for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding human C-Peptide concentration.

- If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent), the concentration read from the standard curve must be multiplied by the dilution factor (x 2).
- Calculation of samples with a concentration exceeding standard 1 will result in incorrect, low human C-Peptide levels (Hook Effect). Such samples require further external predilution according to expected human C-Peptide values with Sample Diluent in order to precisely quantitate the actual human C-Peptide level.
- It is suggested that each testing facility establishes a control sample of known human C-Peptide concentration and runs this additional control with each assay. If the values obtained are not within the expected range of the control, the assay results may be invalid.
- A representative standard curve is shown in Figure 9

Note: Do not use this standard curve to derive test results. Each laboratory must prepare a standard curve for each group of microwell strips assayed.

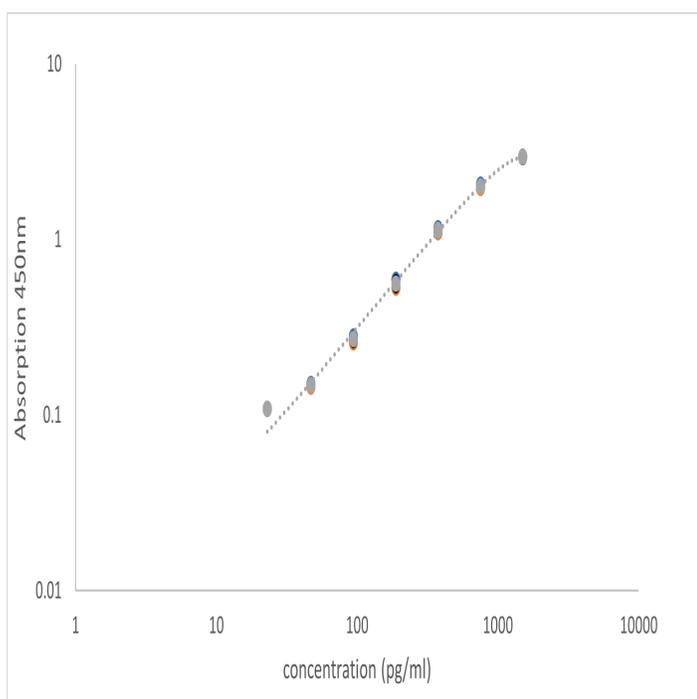


Figure 9 Representative standard curve for human C-Peptide ELISA.
Human C-Peptide was diluted in serial 2-fold steps in Sample Diluent.

Table 2 Typical data using the human C-Peptide ELISA

Standard	Human C-Peptide Concentration (pg/mL)	O.D. at 450 nm ^[1]	Mean O.D. at 450 nm	C.V. (%)
1	1500	2.939 2.979	2.959	1
2	750	2.063 1.963	2.013	3
3	375	1.157 1.106	1.132	3
4	188	0.592 0.535	0.563	7
5	94	0.280 0.261	0.271	5
6	47	0.151 0.146	0.149	2
7	23	0.109 0.109	0.109	1
Blank	0	0.085 0.083	0.084	2

^[1] Measuring wavelength: 450 nm

The OD values of the standard curve may vary according to the conditions of assay performance (e.g., operator, pipetting technique, washing technique, or temperature effects). Furthermore, shelf life of the kit may affect enzymatic activity and thus color intensity. Values measured are still valid.

Limitations

- Since exact conditions may vary from assay to assay, a standard curve must be established for every run.
- Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- Disposable pipette tips, flasks or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Wash Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

Performance characteristics

Sensitivity

The limit of detection of human C-Peptide defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 2.9 pg/mL (mean of 3 independent assays).

Reproducibility

Intra-assay

Reproducibility within the assay was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of serum and plasma samples containing different concentrations of human C-Peptide. 2 standard curves were run on each plate. Data below show the mean human C-Peptide concentration and the coefficient of variation for each sample (see “Intra-assay” on page 7). The calculated overall intra-assay coefficient of variation was 5.5%.

Table 3 The mean human C-Peptide concentration and the coefficient of variation for each sample

Sample	Experiment	Mean Human C-Peptide concentration (pg/mL)	Coefficient of variation (%)
1	1	606.7	13.6
	2	767.6	8.0
	3	648.0	7.9
2	1	386.9	2.4
	2	476.0	3.9
	3	403.8	4.7
3	1	249.9	3.7
	2	306.8	8.4
	3	261.0	4.0
4	1	144.1	3.5
	2	169.0	3.5
	3	151.1	3.8
5	1	69.7	5.0
	2	94.8	17.0
	3	71.4	3.8
6	1	106.2	2.6
	2	137.7	3.1
	3	107.4	2.9
7	1	50.6	4.4
	2	54.2	3.7
	3	51.5	5.9
8	1	69.0	3.6
	2	73.8	4.9
	3	67.6	8.8

Inter-assay

Assay to assay reproducibility within one laboratory was evaluated in 3 independent experiments. Each assay was carried out with 6 replicates of serum and plasma samples containing different concentrations of human C-Peptide. 2 standard curves were run on each plate. Data below show the mean human C-Peptide concentration and the coefficient of variation calculated on 18 determinations of each sample (see Table 4). The calculated overall inter-assay coefficient of variation was 10.5%.

Table 4 The mean human C-Peptide concentration and the coefficient of variation of each sample

Sample	Mean Human C-Peptide concentration (pg/mL)	Coefficient of variation (%)
1	674.1	12.4
2	422.2	11.2
3	272.6	11.1
4	154.7	8.3
5	78.6	17.9
6	117.1	15.2
7	52.1	3.7
8	70.1	4.7

Spike recovery

The spike recovery was evaluated by spiking 2 levels of human C-Peptide into serum, plasma (EDTA and citrate), and cell culture supernatant. Recoveries were determined with 2 replicates each. The amount of endogenous human C-Peptide in unspiked samples was subtracted from the spike values.

Table 5

Sample matrix	Spike high		Spike Low	
	Mean (%)	Range (%)	Mean (%)	Range (%)
Serum	109	102–122	116	105–125
Plasma (EDTA)	80	73–88	100	91–109
Plasma (Citrate)	97	80–126	112	102–122
Cell culture supernatant	108	—	99	—

Dilution parallelism

Serum, plasma (EDTA, citrate), and urine samples with different levels of human C-Peptide were analysed at serial 2 fold dilutions with 2 replicates each.

Sample matrix	Dilution	Recovery of Expected Values	
		Mean (%)	Range (%)
Serum	4	107	98 – 111
	8	112	94 – 130
	16	91	86 – 97
Plasma (EDTA)	4	93	93 – 93
	8	94	86 – 101
	16	70	70 – 70
Plasma (citrate)	4	106	104 – 108
	8	111	104 – 119
	16	93	74 – 113
Urine	8	87	85 – 90
	16	86	81 – 91
	32	84	82 – 85

Sample stability

Freeze-Thaw stability

Aliquots of serum were stored at -20°C and thawed 3 times, and the human C-Peptide levels determined. There was no significant loss of human C-Peptide immunoreactivity detected by freezing and thawing.

Storage stability

Aliquots of serum were stored at -20°C, 2-8°C, room temperature (RT) and at 37°C, and the human C-Peptide level determined after 24 hours. There was no significant loss of human C-Peptide immunoreactivity detected during storage under above conditions.

Specificity

The assay detects both natural and recombinant human C-Peptide. There was no cross reactivity or interference detected.

Expected values

Panels of 40 serum, plasma samples (EDTA and citrate), and urine from randomly selected healthy donors (males and females) were tested for C-Peptide.

Sample matrix	Number of samples evaluated	Mean (pg/mL)	Range (pg/mL)	Standard deviation (pg/mL)
Serum	16	48.2	0.0–151.4	36.5
Plasma (citrate)	8	32.6	13.2–46.5	12
Plasma (EDTA)	8	23.3	8.3–54.3	13.9
Urine	8	2429.9	981.9–3896.9	1165.2

Note: The levels measured may vary with the sample collection used.

Reagent preparation summary

Wash buffer (1X)

Add Wash Buffer Concentrate 20X (50 mL) to 950 mL distilled water.

Number of Strips	Wash Buffer Concentrate (mL)	Distilled Water (mL)
1–6	25	475
1–12	50	950

Assay buffer (1X)

Add Assay Buffer Concentrate 20X (5 mL) to 95 mL distilled water.

Number of Strips	Assay Buffer Concentrate (mL)	Distilled Water (mL)
1–6	2.5	47.5
1–12	5.0	95.0

Biotin-Conjugate

Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with Assay Buffer (1X) in a clean plastic tube as needed according to the following table:

Number of Strips	Biotin-Conjugate (mL)	Assay Buffer (1X) (mL)
1–6	0.06	5.94
1–12	0.12	11.88

Streptavidin-HRP

Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with Assay Buffer (1X) in a clean plastic tube as needed according to the following table:

Number of Strips	Streptavidin-HRP (mL)	Assay Buffer (1X) (mL)
1–6	0.06	5.94
1–12	0.12	11.88

Human C-Peptide standard

Reconstitute human C-Peptide standard with distilled water. (Reconstitution volume is stated on the label of the standard vial.)

Test protocol summary

1. Determine the number of microwell strips required.
2. Wash microwell strips twice with Wash Buffer.
3. **Standard dilution on the microwell plate:** Add 100 µL Sample Diluent, in duplicate, to all standard wells. Pipette 100 µL prepared standard into the first wells and create standard dilutions by transferring 100 µL from well to well. Discard 100 µL from the last wells.

Alternatively **external standard dilution** in tubes (see “External standard dilution” on page 4): Pipette 100 µL of these standard dilutions in the microwell strips.
4. Add 100 µL Sample Diluent, in duplicate, to the blank wells.
5. Add 50 µL Sample Diluent to sample wells.
6. Add 50 µL sample in duplicate, to designated sample wells.
7. Cover microwell strips and incubate 2 hours at room temperature on a microplate shaker.
8. Prepare Biotin-Conjugate.
9. Empty and wash microwell strips 6 times with Wash Buffer.
10. Add 100 µL diluted Biotin-Conjugate to all wells.
11. Cover microwell strips and incubate 1 hour at room temperature.
12. Prepare Streptavidin-HRP.

13. Empty and wash microwell strips 6 times with Wash Buffer.
14. Add 100 µL diluted Streptavidin-HRP to all wells.
15. Cover microwell strips and incubate for about 30 minutes at room temperature on a microplate shaker.
16. Empty and wash microwell strips 6 times with Wash Buffer.
17. Add 100 µL of TMB Substrate Solution to all wells.
18. Incubate the microwell strips for about 30 minutes at room temperature.
19. Add 100 µL Stop Solution to all wells.
20. Blank microwell reader and measure colour intensity at 450 nm.

Note: If instructions in this protocol have been followed, samples have been diluted 1:2 (50 µL sample + 50 µL Sample Diluent) and the concentration read from the standard curve must be multiplied by the dilution factor (x 2).

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Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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Revision	Date	Description
B (31)	17 April 2025	Updated Expected values in performance characteristics.
A.0	9 June 2022	New document for Human C-Peptide ELISA Kit.

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