Catalog #80954

V.2

# C-Peptide ELISA Kit Instructions

For the quantitative determination of
c-peptide in human serum

**Catalog #80954**
**96 Assays**

**For research use only. Not for use in diagnostic procedures.**

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### *A. Intended Use*

The C-Peptide ELISA kit is for the quantitative determination of c-peptide in human serum. Please read the complete kit insert before performing this assay. The kit is for RESEARCH 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. C-peptide is often used in evaluating hypoglycemia and insulinoma.

### *C. Principle of the Assay*

The C-Peptide ELISA kit is an ELISA sandwich assay for c-peptide. It utilizes a specific monoclonal antibody immobilized onto the microplate wells and another monoclonal antibody conjugated with HRP. The sample is incubated in the microplate well with HRP labeled antibody. After incubation, all unbound labeled antibody is removed via a wash step. Subsequently, substrate solution is added and c-peptide levels of the samples can be measured by color intensity.

### *D. Kit Storage*

1. Upon receipt of the 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 |
|---|---|---|
| MIC | Antibody-coated Microplate (12 x 8) | 1 pack |
| STD1-6 | Standards | 6 vials |
| CON1-2 | Controls | 2 vials |
| HRP | HRP Labeled Antibody (Concentrated) | 300 µL/vial |
| AB | Assay Buffer | 1 x 15 mL |
| WASH | Wash Buffer (10X Concentrate) | 1 x 50 mL |
| SUB | Substrate Solution | 1 x 16 mL |
| STOP | Stop Solution | 1 x 6 mL |

#### E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized water
Microplate sealers
Polypropylene microtubes
Volumetric flasks
Microplate shaker (200 rpm)
Microplate reader (capable of reading $A_{450}$ and $A_{630}$ values)

### *F. Assay Precautions*

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

### *G. Maximizing Kit Performance*

1. Given the small sample volumes required (50 µL), pipetting should be done as carefully as possible. A high quality 50 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. Each standard and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

### *H. Sample Collection*

Serum samples can be capped and stored at 2-8°C for up to 24 hours prior to assaying. For longer term storage, samples should be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Thawed samples should be inverted several times prior to testing. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.

### *I. Assay Procedure*

All reagents, unless otherwise noted, are stable till the expiration date at 2-8°C once opened.

#### I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-6
   Standards are provided in liquid form with concentrations ranging from 0 ng/mL to 16 ng/mL. Standards, once opened, are stable for two weeks at 2-8°C. For longer term storage, opened standards should be frozen. Standards should be not be repeatedly thawed, so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. Standards are approximately provided in the following concentrations: 0, 0.3, 0.8, 2, 8, and 16 ng/mL. For exact values, please see calibrator labels on each bottle as values may vary slightly from lot to lot.
3. Controls 1-2
   Controls are provided in liquid form with target value and ranges included on their labels. Controls, once opened, are stable for two weeks at 2-8°C. For longer term storage, controls should be frozen. Controls should be not be repeatedly thawed, so controls should be appropriately aliquoted in appropriate volumes prior to being frozen.

4. HRP Labeled Antibody (Concentrated)
   The HRP labeled antibody must be diluted 1:101 with Assay Buffer prior to use. Please prepare only as needed. For example, mix 20 µL of HRP labeled antibody with 2 mL of Diluent and mix thoroughly. To run the whole plate, 120 µL of HRP labeled antibody should be mixed with 12 mL of Assay Buffer and mixed thoroughly.
5. Assay Buffer
   Provided as ready to use.
6. Wash Buffer (10X Concentrated)
   The wash buffer has to be diluted 1:10 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 450 mL of deionized water. Dilute only as needed.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

### I.2. 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. In each well, add 50 µL of sample, standard, or control and mix well by repeated pipetting.
2. In each well, add 50 µL of working HRP labeled antibody into each well.
3. Incubate the plate for 90 mins at room temperature (shake at 200 rpm).
4. Aspirate well contents and wash three 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.
5. Add 150 µL of Substrate Solution in each well.
6. Incubate the plate for 20 mins in dark room at room temperature.
7. Stop the reaction by adding 50 µL of Stop Solution.
8. Measure absorbance within 20 minutes using a plate reader (measure $A_{450}$ values and subtract $A_{630}$ values).

### I.3. Determining the c-peptide concentration

1. Using computer software, construct the c-peptide calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding c-peptide concentration on the X axis. A four or five parameter curve fit is suitable for the evaluation.
   ***Note:*** *A calibration curve should be plotted every time the assay is performed.*
2. C-peptide concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples, the values obtained must be multiplied by the dilution factor to obtain the final c-peptide concentration. The c-peptide concentration is expressed in ng/mL.
   ***Note:*** *Samples with high c-peptide concentrations (ie. fall above the range of the assay) should be further diluted with the Standard 1 and rerun.*

## *J. Performance characteristics*

### J.1. Assay range

The C-Peptide ELISA Kit has an assay range from 0.3 – 16 ng/mL. The analytical sensitivity of the assay is 0.2 ng/mL.

### J.2. Precision

The assay has a within-run and total precision of CV $\leq$ 10%.

**J.3. Precision**

The table below indicates the analyte and the percent cross reactivity observed in the assay.

**TABLE 2 Cross reactivity**

| Analyte | Cross Reactivity (%) |
|---|---|
| C-Peptide | 100 |
| Insulin | ND |

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