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Instructions for use

C-Peptide ELISA

REF **ME E-0100R**

2°C – 8°C

Σ 96

RUO For research use only – Not for use in diagnostic procedures

C-Peptide ELISA

1 INTENDED USE

The **C-Peptide ELISA** is an enzyme immunoassay for the quantitative measurement of C-Peptide in serum, plasma (EDTA, lithium heparin or citrate plasma) and urine.

1.1 Summary and Explanation

Insulin is synthesized in the pancreatic beta cells as a 6000 MW component of an 86 amino acid polypeptide called proinsulin (1, 2, 3). Proinsulin is subsequently cleaved enzymatically, releasing insulin into the circulation along with a residual 3000 MW fragment called connection ("C") peptide, because it connects A and B chains of insulin within the proinsulin molecule (1, 2, 3, 4). Human C-Peptide, a 31 amino acid residue peptide, has a molecular mass of approximately 3000 daltons. C-Peptide has no metabolic function. However, since C-Peptide and insulin are secreted in equimolar amounts, the immunoassay of C-Peptide permits the quantitation of insulin secretion (4, 5, 6). This is the reason for the interest of serum and urinary determinations of C-Peptide. Moreover, C-Peptide measurement has several advantages over immunoassays of insulin.
The half-life of C-Peptide in the circulation is between two and five times longer than that of insulin (7). Therefore, C-Peptide levels are a more stable indicator of insulin secretion than the more rapidly changing levels of insulin. A very clear practical advantage of C-Peptide measurement arising from its relative metabolic inertness as compared to insulin is that C-Peptide levels in peripheral venous blood are about 5 – 6 times greater than insulin levels (3). Also, relative to an insulin assay, the C-Peptide assay's advantage is its ability to distinguish endogenous from injected insulin.
C-Peptide levels are in many ways a better measurement of endogenous insulin secretion than peripheral insulin levels. C-Peptide may be measured in either blood or urine (9). With improved sensitive C-Peptide immunoassays, it is now possible to measure C-Peptide values at extremely low levels.

2 PRINCIPLE OF THE TEST

The C-Peptide ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the **principle of competitive binding.**
The microtiter wells are coated with anti-mouse antibodies, which in turn bind monoclonal antibodies directed against an epitope of the C-peptide molecule.
During an incubation, C-Peptide in the added sample competes with the added enzyme conjugate, which is C-Peptide conjugated to horseradish peroxidase, for the free binding sites on the immobilised antibodies.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of colour is inversely proportional to the concentration of the analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C – 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution coloured. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (20 °C – 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.

15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

4 REAGENTS

4.1 Reagents provided

1. ME E-0131 **MTP 96** **Microtiterwells**

Content: 12x8 (break apart) strips, 96 wells;
Wells coated with anti-mouse-antibody

2. Standards – lyophilized

Cat. no.	Component	Standard	Concentration	Volume/Vial
ME E-0101	**STANDARD A**	Standard A	0 – 16 ng/ml (see exact value on the vial label or on the QC-Datasheet)	0.75 ml
ME E-0102	**STANDARD B**	Standard B		0.75 ml
ME E-0103	**STANDARD C**	Standard C		0.75 ml
ME E-0104	**STANDARD D**	Standard D		0.75 ml
ME E-0105	**STANDARD E**	Standard E		0.75 ml
ME E-0106	**STANDARD F**	Standard F		0.75 ml

The standards are calibrated against WHO approved International Reference Reagent IRR C-Peptide, NIBSC code 84/510.
*Conversion: 1 ng/ml * 0.33 = 1 nmol/l*
See „Preparation of Reagents";
Contain non-mercury preservative.

3. ME E-0160 **SAMPLE-DIL** **Sample Diluent** – ready to use

Content: 1 vial, 3 ml;
Contains non-mercury preservative.

4. ME E-0110 **AS** **Antiserum** – ready to use

Content: 1 vial, 7 ml;
Monoclonal mouse anti C-Peptide antibody;
Contains non-mercury preservative.

5. ME E-0140 **CONJUGATE** **Enzyme Conjugate** – ready to use

Content: 1 vial, 14 ml;
Biotinylated C-Peptide;
Contains non-mercury preservative.

6. ME E-0141 **ENZYME** **Enzyme Complex** – ready to use

Content: 1 vial, 14 ml;
Contains horseradish Peroxidase;
Contains non-mercury preservative.

7. FR E-0055 **SUBSTRATE** **Substrate Solution** – ready to use

Content: 1 vial, 14 ml;
TMB

8. FR E-0080 **STOP-SOLN** **Stop Solution** – ready to use

Content: 1 vial, 14 ml;
Contains 0.5 M H_2SO_4;
Avoid contact with the Stop Solution. It may cause skin irritations and burns.

9. FR E-0030 **WASH-CONC** **40x** **Wash Solution** – 40X concentrated

Content: 1 vial, 30 ml;
See „Preparation of Reagents"

10. Instructions for Use

11. QC-Datasheet

4.2 Materials required but not provided

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Manual or automatic equipment for rinsing microtiter plates
- Absorbent paper
- Distilled water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 Storage Conditions

When stored at 2 °C – 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2 °C – 8 °C. Microtiter wells must be stored at 2 °C – 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 2 months if stored as described above.

4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

Standards
Reconstitute the lyophilized contents of each standard vial with 0.75 ml distilled water and let stand for 10 minutes in minimum. Mix several times before use.

Note: The reconstituted standards are stable for 3 days at 2 °C – 8 °C.
For longer storage the reconstituted standards should be aliquoted and stored at -20 °C.

Wash Solution
Add distilled water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated *Wash Solution* with 1170 ml distilled water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 1 week at room temperature.

4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN COLLECTION AND PREPARATION

Serum, plasma (EDTA, lithium heparin or citrate plasma) or urine can be used in this assay.

Note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use haemolytic, icteric, or lipaemic specimens. For further information refer to chapter "*Interfering Substances*".

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Donors receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Urine:
The total volume of urine excreted during a 24-hour-period should be collected and mixed in a single container.

Note: Specimens should be stored at 2 °C – 8 °C during collection period and total volume collected should be recorded.

5.2 Specimen Storage and Preparation

Serum / Plasma:
Specimens must be capped and can be stored for up to 7 days (serum) or 2 days (plasma) at 2 °C 8 °C prior to performing the assay. (23)
Specimens held for a longer time (up to 1 month) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

Urine:
Aliquot a well-mixed sample to be used in the assay. Centrifuge sample to clear. Urine samples may be stored for up to 36 hours at 2 °C – 8 °C prior to assaying.
Specimens held for a longer time (up to 12 months) should be frozen only once at -20 °C prior to assay.

5.3 Specimen Dilution

Serum / Plasma Samples
If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Sample Diluent* and re-measured as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.

Example:
a) dilution 1:10: 10 µl sample + 90 µl *Sample Diluent* (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl *Sample Diluent* (mix thoroughly).

Urine Samples
Prior to use dilute urine samples **1:20** with *Sample Diluent*. The results must therefore be multiplied by a dilution factor of 20.

If the Sample Diluent included in the kit is insufficient, you can order additional *Sample Diluent* (40 ml).

6 ASSAY PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Test Procedure

Each run must include a standard curve.

1.	Secure the desired number of Microtiter wells in the frame holder.
2.	Dispense **100 µl** of each *Standard*, control and sample with new disposable tips into appropriate wells.
3.	Dispense **50 µl** *Antiserum* into each well.
4.	Dispense **100 µl** *Enzyme Conjugate* into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5.	Incubate for **60 minutes** at room temperature with shaking (500 – 600 rpm).
6.	Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets. **Important note:** The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7.	Add **100 µl** of *Enzyme Complex* to each well.
8.	Incubate for **30 minutes** at room temperature with shaking (500 – 600 rpm).
9.	Briskly shake out the contents of the wells. Rinse the wells 3 times with diluted Wash Solution (400 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets.
10.	Add **100 µl** of *Substrate Solution* to each well.
11.	Incubate for **20 minutes** at room temperature.
12.	Stop the enzymatic reaction by adding **100 µl** of *Stop Solution* to each well.
13.	Measure the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader. It is recommended that the wells be read **within 10 minutes** after adding the *Stop Solution*.

6.3 Calculation of Results

1. Calculate the average optical density (OD) values for each set of standards, controls and samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 16 ng/ml. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Density (450 nm)
Standard A (0.0 ng/ml)	1.82
Standard B (0.2 ng/ml)	1.64
Standard C (0.7 ng/ml)	1.46
Standard D (2.0 ng/ml)	1.02
Standard E (6.0 ng/ml)	0.47
Standard F (16.0 ng/ml)	0.21

7 REFERENCE RANGES HEALTHY

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the C-Peptide ELISA the following values are observed:

	n	Mean ± 2SD
Serum (Post 12-hour Fasting)	60	0.50 – 3.20 ng/ml

	n	Range (min. – max.)	Mean	Median	2.5th Percentile	97.5th Percentile
Serum (females < 50 years)	42	< 0.174 – 5.83 ng/ml	1.02 ng/ml	0.71 ng/ml	0.18 ng/ml	4.06 ng/ml
Urine (males and females)	10	0.90 – 200 µg/day	107.83 µg/day	116.00 µg/day	3.06 µg/day	199.10 µg/day

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above-mentioned items without finding any error contact your distributor or the manufacturer directly.

9 PERFORMANCE CHARACTERISTICS

9.1 Specificity of Antibodies (Cross Reactivity)

Substance	Concentration added (ng/ml)	Cross-Reactivity %
Proinsulin	7.30	3.8
Insulin	3.40	< 1.5
IGF-1	300.00	< 0.02

9.2 Detection Capability

Limit of Blank (LoB)	0.04 ng/ml
Limit of Detection (LoD)	0.174 ng/ml
Limit of Quantification (LoQ)	0.401 ng/ml
Lower Limit of Linear Interval (LLLI)	0.410 ng/ml
Measuring range	0.174 ng/ml – 16 ng/ml
Linear range	0.410 ng/ml – 13.30 ng/ml

9.3 Repeatability and Reproducibility

9.3.1 Repeatability (Within-Run Precision)

The within assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	20	0.48	6.5
2	20	2.30	6.7
3	20	3.86	5.1

9.3.2 Reproducibility (Between-Run)

The between assay variability is shown below:

Sample	n	Mean (ng/ml)	CV (%)
1	12	0.42	9.3
2	12	2.05	9.9
3	12	4.23	8.4

9.3.3 Reproducibility (Between-Lot)

The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 3 different kit lots:

Sample	n	Mean (ng/ml)	CV (%)
1	21	1.86	4.8
2	21	5.05	3.2
3	21	13.9	2.9

9.4 Recovery

Samples have been spiked by adding C Peptide solutions with known concentrations.
The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

Sample		**1**	**2**	**3**	**4**	**5**	**6**
Sample type		**Serum**	**Serum**	**Serum**	**Urine**	**Urine**	**Urine**
Concentration (ng/ml)		5.36	9.70	12.12	0.34	1.45	1.58
Average Recovery (%)		98.7	94.3	102.3	95.3	96.9	89.6
Range of Recovery (%)	from	96.5	87.3	88.1	85.4	88.7	85.4
	to	101.7	104.8	110.4	106.4	105.5	100.1

9.5 Linearity

Samples were measured undiluted and in serial dilutions with Standard A. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

Sample		**1**	**2**	**3**	**4**	**5**	**6**
Sample Type		**Serum**	**Serum**	**Serum**	**Urine**	**Urine**	**Urine**
Concentration (ng/ml)		6.10	9.90	13.25	8.70	9.20	13.90
Average Recovery (%)		107.6	107.2	102.0	97.1	99.1	97.9
Range of Recovery (%)	from	105.3	100.2	97.1	92.4	97.4	95.0
	to	110.6	112.8	105.1	100.2	102.2	103.6

10 LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.
Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.
A biotin concentration of up to 1200 ng/ml in a sample has no influence on the assay results.

10.2 Drug Interferences

Until today, no substances (drugs) are known to us, which have an influence on the measurement of C-Peptide in a sample.

10.3 High-Dose-Hook Effect

A High-Dose-Hook Effect is not known for competitive assays.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover, the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include a sufficient number of external controls in the test procedure in order to validate the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

11.2 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2 are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES / LITERATURE

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Symbols:

+2 +8 °C	Storage temperature		Manufacturer	Σ	Contains sufficient for <n> tests
	Use-by date	LOT	Batch code		
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		Distributor
	Date of manufacture			RUO	For research use only!