

## hC-PEPTIDE [I-125] IRMA KIT (REF: RK-84CT)

The C-PEPTIDE [<sup>125</sup>I] IRMA system provides a direct quantitative determination of C-peptide in human serum and urine. C-peptide can be assayed in the range of 0-30 ng/ml (in human serum) and 0-300 ng/ml (in urine) using 50 µl serum samples. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.

### Introduction

C-peptide (connective peptide) is a polypeptide with a molecular weight of 3600, containing 31 amino acid. It derives from proinsulin produced in pancreatic beta cells. Proinsulin is converted to insulin and C-peptide, which are then secreted into the portal blood in an equimolar quantity. Contrary to insulin, C-peptide is not extracted by the liver, but enters the systemic circulation.

In the diagnosis of diabetes, C-peptide is a more reliable indicator of insulin secretion than is the insulin itself. Furthermore, the concentration of C-peptide is not affected by interference from insulin antibodies often present in patients receiving insulin therapy. The assessment of residual endogenous insulin secretion by the measurement of C-peptide is an indispensable tool for the diagnosis and treatment of diabetes mellitus.

### Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system. The <sup>125</sup>I labelled signal-antibody binds to an epitope of the C-peptide molecule, which is different from that recognised by the unlabelled capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich". During a 3-hour incubation period with continuous agitation immuno-complex is immobilised on the reactive surface of test tubes. Reaction mixture is then discarded, test tubes are washed exhaustively, and the radioactivity is measured in a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of standards containing known amount of C-peptide, the unknown concentration of C-peptide in patient samples can be determined.

### Contents of the kit

1. 1 bottle of TRACER, 21 ml, ready to use. Contains less than 740 kBq of <sup>125</sup>I labelled anti-C-peptide and biotin labelled anti-C-peptide in buffer containing proteins, 0.1% sodium azide, red coloured.

2. 6 vials of STANDARDS (S0-S5), S0 ready to use: 2.5 ml, bovine serum containing 0.1% NaN<sub>3</sub>. (S1-S5) lyophilized: 0.5 ml/vial, human serum containing 0.1% NaN<sub>3</sub>.

Conc.: 0, 0.25, 0.9, 3, 9, 30 ng/ml.

3. 1 vial of CONTROL SERUM, lyophilized. 0.5 ml human serum, containing 0.1% NaN<sub>3</sub>. *The concentration of control serum is specified in the quality certificate enclosed.*

4. 2 boxes of COATED TUBES, ready to use. 2x50 plastic tube, coated with streptavidin.

5. 1 bottle of WASH BUFFER CONCENTRATE, 20 ml, with 0.2% NaN<sub>3</sub>.

Quality certificate

Pack leaflet

### Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips 20µl (if urine is assayed), 50µl 200µl, 1.5ml, shaker, plastic foil, adsorbent tissue, gamma counter

**Recommended tools and equipment** repeating pipettes, dispenser with reservoir (instead of the 1.5-ml pipette)

### Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Do not store serum samples longer than 2 months. Do not use lipemic, hemolyzed or turbid specimens. Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided.

24-hour urine should be collected by an immediate freezing, then combining, the daily portions taken. Do not store urine samples longer than 2 months. For use in the assay, frozen urine should be thawed, mixed thoroughly, and diluted 10-fold. Dilute 20 µl urine with 180 µl zero standard.

### Preparation of reagents, storage

Store the reagents between 2-8°C after opening. At this temperature reagent (except reconstituted standard and control) is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.

Add 0.5 ml distilled water to the *lyophilised standard and control serum*, and mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. For repeated use the rest of reagent can be stored at -20 °C for two months. It is not recommended to expose the same standard vial to more than two freeze-melting cycles.

Add the *wash buffer concentrate* to 700 ml distilled water. The diluted solution can be stored at 2-8 °C until expiry date of the kit.

**CAUTION!** Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

### Assay procedure

(For a quick guide)

1. Label coated tubes in duplicate for each standard (S0-S5), control serum(C) and samples(P). Optionally, label two test tubes for total count (T).
2. Pipette **50 µl** each of STANDARD(S0-S5), CONTROL(C) and SAMPLES(P) into the properly labelled tubes.
3. Pipette **200 µl** of TRACER into each tube.
4. Gently vortex all tubes. Seal all tubes with a plastic foil. If optional total counts tubes are also prepared, place them separately from others.
5. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube. Incubate tubes for 3 hours at room temperature. (*Note: The efficient rotation is a critical factor to achieve good performance. An uneven or incomplete shaking may result in a serious error. Never use a rack type with open hole.*)
6. Add 1.5 ml diluted wash buffer to each tube and decant the supernatant from all tubes by the inversion of the rack. In the upside down position place the rack on an absorbent paper for 2 minutes.
7. Repeat Step 6.
8. Repeat Step-6. (*The third washing step can be omitted. See notes on the effect of this option later.*)
9. Count each tube for at least 60 seconds in a gamma counter.

### Calculation of results

Calculate the average CPM for each pair of assay tubes. Draw the standard curve by plotting mean CPM of each standard level (ordinate) against the respective concentration, except for 0 standard (abscissa) using log-log graph paper.

Obtain sample concentration by interpolation of sample counts on the standard curve.

For computerised calculations and/or quality assessment normalised specific binding values, rather than cpm values are used. Specific binding values can be calculated for each standard and sample according to the following equation:

$$B/T\% = \frac{S1-S5, C, P(\text{cpm}) - S0(\text{cpm})}{T(\text{cpm})} \times 100$$

#### Assay Protocol, Pipetting Guide

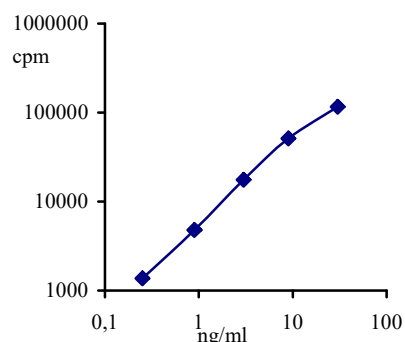
(all volumes in microlitres)

	T	S0-S5	C	P
Standard		<b>50</b>		
Control			<b>50</b>	
Samples				<b>50</b>
Tracer	<b>200</b>	<b>200</b>	<b>200</b>	<b>200</b>
Vortex mix				
Rotate for 3 hours at room temperature				
Wash buffer		<b>1500</b>	<b>1500</b>	<b>1500</b>
Decant the fluid and blot on filter paper				
Wash buffer		<b>1500</b>	<b>1500</b>	<b>1500</b>
Decant the fluid and blot on filter paper				
Wash buffer		<b>1500</b>	<b>1500</b>	<b>1500</b>
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

### Typical assay data

Tubes	Count cpm	Mean cpm	Bo/T %	ng/ml
T	273835 274213	274024		
S0	74 68	71	0.00	
S1	1363 1378	1370	0.47	
S2	4744 4815	4780	1.72	
S3	17158 18023	17591	6.39	
S4	50937 53632	52285	19.05	
S5	115771 118580	117176	42.74	
C	27956 26006	26981		4.5

### Typical standard curve



## Characterization of assay

Performance parameters have been determined under ideal experimental conditions; by using fresh tracers, and a 3-step washing protocol. The use of a 2-step washing procedure will only affect the analytical sensitivity.

### Calibration

Standards are calibrated against the WHO Ref. Prep., Code 84/510.

### Assay parameters

NSB/T < 0.05% (< 0.08 % with 2-step wash)  
 $B_{\max}/T > 35\%$

### Analytical sensitivity

The analytical sensitivity is 0.0056 ng/ml (0.0186 with the 2-step washing procedure). It is defined as the concentration of C-peptide equivalent to the mean CPM of 20 replicates of the zero standard.

### Functional sensitivity

The value of functional sensitivity is found to be 0.105 ng/ml.

It is defined as the value extrapolated to 20 % of the inter-assay imprecision profile obtained from 10 independent runs on patient samples with low endogenous C-peptide concentration

### Hook effect

There is no high dose "hook effect" up to a C-peptide concentration of 90 ng/ml.

### Specificity

Cross reactivity values are defined as the mass ratio of cross-reacting agent versus C-peptide, belonging to the same binding rate. Based on the ratio proinsulin/insulin under physiologic conditions (about 5 %), the potential error contributed by proinsulin must be less than 2-4 %.

Cross-reacting substance	% cross-reaction
Human insulin	< 0.04
Proinsulin	27.1

### Precision and reproducibility

7 samples with 20 replicates in 1 assay run, and with duplicates in 12 runs were measured to determine intra-assay and inter-assay precision, respectively. Values obtained are shown below

Intra-assay		Inter-assay	
mean (ng/ml)	CV %	Mean (ng/ml)	CV %
0.52	4.87	0.52	3.31
1.55	2.12	1.41	4.65
2.33	5.03	3.03	4.07
3.81	3.56	3.10	5.88
5.64	2.09	4.36	2.28
7.57	5.09	6.57	3.25
23.46	4.04	11.96	3.96

### Recovery

Recovery was defined as the measured increase expressed as per cent of expected increase upon spiking serum samples with known amount of C-peptide. Values for 9 serum pools spiked with C-peptide at 3 levels were as follows:  $97.2 \pm 8.5\%$

### Dilution test

4 samples were measured in a series of dilution (2, 4, 8, 16-fold) with zero-standard. The following equation obtained for measured (Y) versus expected (X) concentration demonstrates the good linearity:

$$y = 1.014x - 0.011 \quad R = 0.9973, \quad n = 16$$

### Expected values

It is recommended that each laboratory establish its own reference intervals.

-morning sera, fasting healthy donors (N=27)

$1.77 \pm 0.62 \text{ ng/ml}$  (min. 1.07– max. 3.51 ng/ml)

- daily sera, healthy donors (N=71)

$4.88 \pm 2.6 \text{ ng/ml}$ , (min. 1.37– max. 11.8 ng/ml)

-morning urine, fasting healthy donors (N=29)

$35.8 \pm 19.2 \text{ ng/ml}$ , (min. 2.72 – max. 78.3 ng/ml)

Results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

### Conversion of values

1 nmol/l = 3.617 ng/ml

1 ng/ml = 0.276 nmol/l

## Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

### Precaution

#### Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

## Biohazard








Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative for the presence of antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*. Bovine components originate from countries where bovine spongiform encephalopathy has not been reported. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*. All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

### Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 66 mg.

### Storage and shelf life

Store this product at a temperature of 2-8°C  
 Shelf-life: 60 days from availability.

	Used by	<input type="text" value="LOT"/>	Batch code
	Temperature limitation	<input type="text" value="CONTROL"/>	Control
	Caution, consult accompanying documents	<input type="text" value="CAL"/>	Standard
	Biological risks	<input type="text" value="CT"/>	Coated Tube
	Consult instructions for use	<input type="text" value="TRAC"/>	Tracer
<input type="checkbox" value="IVD"/>	In vitro diagnostic device	<input type="text" value="WASHB"/>	Washing Buffer
	Manufacturer	<input type="text" value="REF"/>	Catalogue number
	Radioactive material		



WEB site: <http://www.izotop.hu>

Technical e-mail: [immuno@izotop.hu](mailto:immuno@izotop.hu)

Commercial e-mail: [commerce@izotop.hu](mailto:commerce@izotop.hu)

**IZOTOP**

INSTITUTE OF ISOTOPES Ltd.

1535 Budapest. Pf.: 851.

Tel.: (+36)1-392-2577, Fax: (+36)1-395-9247

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