

## C-PEP-RIA-CT BL-30-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles - Belgium

Read entire protocol before use C-PEP II-RIA-CT

### I. INTENDED USE

Radioimmunoassay for the in vitro quantitative measurement of human C-Peptide in serum.

### II. GENERAL INFORMATION

- A. Name : Bio-Line **C-PEP-RIA-CT** Kit
- B. Catalogue number : BL-30-CT : 100 tests
- C. Manufactured by : Bio-Line S.A.  
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### III. CLINICAL BACKGROUND

#### A. Biological Activity

Insulin is synthesized in the beta-cells of the islets of Langerhans as a precursor molecule, proinsulin. In the secretory granules of the beta-cells, proinsulin is cleaved into insulin and into a 31-amino-acid peptide, called the Connecting Peptide or C-Peptide. Insulin and C-Peptide are secreted in equimolar amounts. However, because of its longer half-life, the plasma concentration of C-peptide is higher than that of insulin. The determination of plasma C-Peptide allows an assessment of the endogenous insulin production, even in the presence of exogenous insulin administration or in the presence of circulating anti-insulin antibodies. Moreover, the determination of C-Peptide in urine provides a reliable index of the insulin production when blood sampling is difficult or when an integrated estimation of C-Peptide secretion over a period of several hours is requested.

#### B. Clinical applications

- . Assessment of residual beta-cell function in diabetics under insulin therapy
- . Detection and monitoring of the remission phase of type I diabetes
- . Adjunct in the differential diagnosis between type I (insulin-dependent) and type II (non-insulin-dependent) diabetes
- . Diagnosis of insulin-induced factitious hypoglycaemia
- . Contribution to the diagnosis of insulinoma (insulin suppression test)
- . Prognostic index of foetal outcome in pregnant diabetic women
- . Evaluation of insulin secretion in liver disease
- . Monitoring of pancreatectomy

### IV. PRINCIPLES OF THE METHOD

A fixed amount of  $^{125}\text{I}$  labelled Tyr-C-Peptide competes with the C-Peptide to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography is required. After 3 hours incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 3 ml of wash solution and aspirated again. A calibration curve is plotted and the C-Peptide concentrations of the samples are determined by dose interpolation from the calibration curve.


## V. REAGENTS PROVIDED

Tubes coated with anti C-Peptide

Ag	<sup>125</sup> I
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CAL	0
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CAL	N
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Reagents	100 Test Kit	Colour Code	Reconstitution
	2 x 50	grey	Ready for use
TRACER: <sup>125</sup> Iodine labelled Tyr-C-Peptide (HPLC grade) in phosphate buffer with bovine gelatin and azide (<0.1%)	1 vial lyophilised 175 kBq	red	Add 6 ml distilled water
Zero Calibrator in human serum and thymol	1 vial lyophilised	yellow	Add 3 ml distilled water
Calibrators - N = 1 to 5 (see exact values on vial labels) in human serum and thymol	5 vials lyophilised	yellow	Add 1 ml distilled water
<b>WASH SOLN</b> CONC Wash solution (TRIS-HCl)	1 vial 10 ml	brown	Dilute 70 x with distilled water (use a magnetic stirrer).
Controls - N = 1 or 2 <b>in human serum with thymol</b>	2 vials lyophilised	silver	Add 1 ml distilled water

- Note :**
1. Use the zero calibrator for sera dilutions.
  2. 1 ng of the calibrator preparation is equivalent to 1 ng MRC 84/510

## VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl, 100 µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Disposable polystyrene tubes (12 x 75 mm)
4. Vortex mixer
5. Magnetic stirrer
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring <sup>125</sup>I may be used (minimal yield 70%).

## VII. REAGENT PREPARATION

- A. Calibrators:** Reconstitute the zero calibrator with 3.0 ml distilled water and the other calibrators with 1.0 ml distilled water.
- B. Controls:** Reconstitute the controls with 1.0 ml distilled water.
- C. Tracer:** Reconstitute the tracer with 6.0 ml distilled water.
- D. Working Wash solution:** Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day

## VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators, controls and tracer are very unstable, use them immediately after reconstitution. For longer storage periods, aliquots should be made and kept at -20°C for maximally 3 months. Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

## IX. SPECIMEN COLLECTION AND PREPARATION

- Serum samples must be kept at 2-8 °C.
- If the test is not run within 8 hrs, storage in aliquots at -20 °C is recommended.
- Avoid subsequent freeze-thaw cycles.

## X. PROCEDURE

### A. Handling notes

Do not use the kit or components beyond expiry date.

Do not mix materials from different kit lots.

Bring all the reagents to room temperature prior to use.

Thoroughly mix all reagents and samples by gentle agitation or swirling.

Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. High precision pipettes or automated pipetting equipment will improve the precision.

Respect the incubation times.

Prepare a calibration curve for each run, do not use data from previous runs.

### B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
2. Briefly vortex calibrators, controls and samples and dispense 100 µl of each into the respective tubes.  
**This operation must be achieved within 15 minutes.**
3. Dispense 50 µl of <sup>125</sup>Iodine labelled Tyr-C-Peptide into each tube, including the uncoated tubes for total counts.
4. Shake the tube rack gently by hand to liberate any trapped air bubbles.
5. Incubate for 3 hours at room temperature.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 3 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
8. Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
9. Count tubes in a gamma counter for 60 seconds.

## XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

3. Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B<sub>0</sub>(%)) values for each calibrator point as a function of the C-Peptide concentration of each calibrator point. Reject obvious outliers.
4. Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
5. By interpolation of the sample (B/B<sub>0</sub> (%)) values, determine the C-Peptide concentrations of the samples from the calibration curve.
6. For each assay, the percentage of total tracer bound in the absence of unlabelled C-Peptide (B<sub>0</sub>/T) must be checked.

## XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

C-Peptide		cpm	B/Bo (%)
Total count		75295	
Calibrator	0.0 pmol/ml	17690	100.0
	0.09 pmol/ml	14319	80.9
	0.29 pmol/ml	11618	65.7
	0.95 pmol/ml	6534	36.9
	2.98 pmol/ml	3361	19.0
	9.94 pmol/ml	1379	7.8

## XIII. PERFORMANCE AND LIMITATIONS

### A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.04 pmol/ml.

### B. Specificity

The percentage of cross-reaction estimated by comparison of the concentration yielding a 50% inhibition are respectively:

Compound	Cross-Reactivity (%)
Biosynthetic human Proinsulin	5.6%
Human Glucagon	-
Human Insulin	-

### C. Precision

#### INTRA-ASSAY PRECISION

#### INTER-ASSAY PRECISION

Serum	N	<X> ± SD (pmol/ml)	CV (%)	Serum	N	<X> ± SD (pmol/ml)	CV (%)
A	24	0.18 ±	5.6	A	22	0.17 ± 0.02	11.8
B	24	0.01	10.7	B	21	0.28 ± 0.03	10.7
C	24	0.28 ±	5.9	C	22	0.65 ± 0.05	7.7
D	26	0.03	3.3	D	21	1.12 ± 0.11	9.8
E	24	0.68 ±	6.9	E	22	1.68 ± 0.12	7.1

SD: Standard Deviation; CV: Coefficient of variation

### D. Accuracy

#### DILUTION TEST

Sample	Dilution	Theoretical Concent. (pmol/ml)	Measured Concent. (pmol/ml)
Serum	1/1	-	6.99
	1/2	3.50	3.04
	1/4	1.75	1.56
	1/8	0.87	0.80
	1/16	0.44	0.46
	1/32	0.22	0.28
	1/64	0.11	0.07

Samples were diluted with zero calibrator.

## RECOVERY TEST

Sample	added C-Peptide (pmol/ml)	Recovered C-Peptide (pmol/ml)	Recovered (%)
Serum	0.14	0.14	100
	0.19	0.17	112
	0.22	0.22	102
	0.44	0.39	113
	1.12	1.14	98
	3.02	3.14	96

Conversion factor : From ng/ml to pmol/ml : x 3

### E. Time delay between last calibrator and sample dispensing

As shown hereafter, the dispensing of samples must be done within a maximum delay of 15 minutes after the calibrator dispensing.

### TIME DELAY

Serum pmol/ml	0'	5'	10'	15'	20'	30'
C1	0.66	0.53	0.55	0.61	0.52	0.41
C2	2.27	2.14	2.58	1.90	1.79	2.07

## XIV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises.

## XV. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

In a group of 79 normal subjects, the mean human C-Peptide concentration found was 1.02 pmol/ml (range, based on 2.5% to 97.5% percentiles: 0.59 - 1.56 pmol/ml).

## XVI. PRECAUTIONS AND WARNINGS

### Safety

For *in vitro* diagnostic use only.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area, away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

## XVII. BIBLIOGRAPHY

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## XVIII. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS $\mu$ l	CALIBRATORS $\mu$ l	SAMPLE (S) CONTROLS $\mu$ l
Calibrators (0 to 5)	-	100	-
Samples, Controls	-	-	100
Tracer	50	50	50
Incubation	3 hours at room temperature		
Separation	-	Aspirate (or decant)	
Working Wash solution		3.0 ml	
Separation		Aspirate (or decant)	
Counting	Count tubes for 60 seconds		