IVD

C-PEPTIDE (C-P) CHEMILUMINESCENCE IMMUNOASSAY KIT

Catalog No. CL1202-2

INTENDED USE

The Autobio C-peptide (C-P) Chemiluminescence Immunoassay (CLIA) kit is intended for the quantitative determination of C-P concentration in human serum.

INTRODUCTION

Insulin is a member of structurally related regulatory proteins; other proteins in this group include the insulin-like growth factors and relaxin. Insulin is produced by the -cells of the pancreatic islets and is initially synthesized as a 12kDa preprohormone, which undergoesintracellular processing to a 9 kDa, 86-amino acid prohormone and subsequent packaging in storage granules. Within these granules, disulfide bonds are formed between the A and B chains of the insulin molecule and the C-peptide region is cleaved, resulting in the 51-amino acid, 6 kDa mature insulin molecule. Upon stimulation, the islet cells release equimolar amounts of insulin and C-peptide, and small amounts of proinsulin and other intermediates(<5% of mormal total insulin secretion).

Basal and glucose-stimulated circulating insulin concentrations are relatively stable during infancy and childhood, and increase during puberty due to decreased insulin sensitivity. Insulin concentrations tend to be higher in obese individuals, particularly those with an increased proportion of visceral (abdominal) fat. Glucose counter-regulatory hormones, such as glucagons, gluconcorticoids, growth hormone and epinephrine, decrease insulin sensitivity and action; insulin levels may increase during exogenous administration of these substances.

Measurement of circulating insulin concentrations may be useful in the diagnostic evaluation of several conditions. Elevated serum insulin levels in the presence of low glucose concentrations may be indicative of pathologic hyperinsulinism, e.g. nesidioblastosis and islet-cell tumor. Elevated serum fasting insulin levels with normal or elevated glucose concentrations, and exaggerated insulin and glucose response to exogenous glucose administration are characteristic of the insulin-resistant forms of glucose intolerance and diabetes mellitus and other insulin resistant conditions. High circulating insulin concentrations may be involved in the pathogenesis of hypertension and cardiovascular disease. Conversely, low insulin concentrations in the presence of hyperglycemia suggest insulin-deficiency, e.g. insulin-dependent or Type Ⅰdiabetes mellitus. Measurement of immediate or first-phase insulin secretion after an acute glucose load may be predictive of Type Ⅰdiabetes mellitus.

Although the c-peptide of insulinis biologically inactive, it has a longer circulating half-life than insulin and undergoes relatively minimal hepatic metabolism. In addition, c-peptide of insulin assays may be analytically more sensitive than insulin assays. Because of these factors,measurements of c-peptide of insulin may be useful in evaluating insulin secretion in a variety of clinical conditions.

PRINCIPLE OF THE TEST

The C-P CLIA test is a solid phase two-site immunoassay. One monoclonal antibody is coated on the surface of the microtiter wells and another monoclonal antibody labeled with horseradish peroxidase is used as the tracer. The C-P molecules present in the standard solution or serum are "sandwiched" between the two antibodies. Following the formation of the coated antibody-antigen-antibody-enzyme complex, the unbound antibody enzyme labels are removed by washing. The horseradish peroxidase activity bound in the wells is then assayed by chemiluminescence reactions. The Related Light Unit (RLU) of the reaction is proportional to the concentration of C-P present in the sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with monoclonal antibodies to C-peptide (anti-C-P MAb) coated wells (1 plate, 48wells/96 wells)
2. Enzyme Conjugate Reagent: Horseradish peroxidase (HRP) labeled anti-C-P MAb in Stabilizing Buffer (1 vial, 3.0ml/6.0ml)
3. Reference Standards: 0.6, 1.2, 2.4, 5, 11ng/ml. (5 vials, Lyophilized)
4. Substrate A (1 vial, 3.5ml/6.0ml)
5. Substrate B (1 vial, 3.5ml/6.0ml)

6. PBS-T powder: PBS-Tween (1bag, 5g)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit.

1. Precision pipettes and tips, 0.05ml, 0.1ml, 1.0ml
2. Distilled water.
3. Vortex mixer
4. Magnetic stirrer
5. Absorbent paper or paper towel
6. Graph paper
7. Luminometer

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2 ～ 8℃ upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Blood should be drawn using standard venipuncture techniques and the serum should be separated from the red blood cells as soon as practical. Avoid grossly hemolytic, lipemic or turbid samples.
2. Plasma samples collected in tubes containing EDTA, heparin, or oxalate may interfere with test procedures and should be avoided.
3. Specimens should be capped and may be stored up to 48 hours at 2 ～ 8℃, prior to assaying. Specimens held for a longer time can be frozen at -20℃. Thawed samples must be mixed prior to testing. Multiple freeze-thaw cycles should be avoided.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. The reference satandards contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, the reference satandards and components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents.
5. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18 ～ 25℃) before use. All reagents should be mixed through gently inverting or swirling prior to use. Do not induce foaming.
2. To prepare washing buffer: add 1 bag of washing buffer concentration to 500ml of distilled water, and mix well. The washing buffer is stable at room termperature at least for two weeks.

IMPORTANT NOTES

1. The wash procedure is critical. insufficient washing will result in poor precision and falsely elevated RLU values.
2. It is recommended that no more than 32 wells be used for each assay run, if manual pipetting is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of all standards and specimens, although not required, is recommended.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense 50μl of reference standards, specimens, and controls into appropriate wells.
2. Dispense 50μl of Enzyme Conjugate Reagent to each well. Mix gently for 30 seconds.

3. Incubate at 37℃ for 60 minutes.
4. At the end of the incubation, remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with washing buffer. Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets. The volume of each well is about 350μl.
5. Dispense 50μl substrate A, then 50μl substrate B into each well. Gently mix for 10 seconds.
6. Incubate at room temperature in the dark for 5 minutes without shaking and read the RLU values with a Luminolmeter.

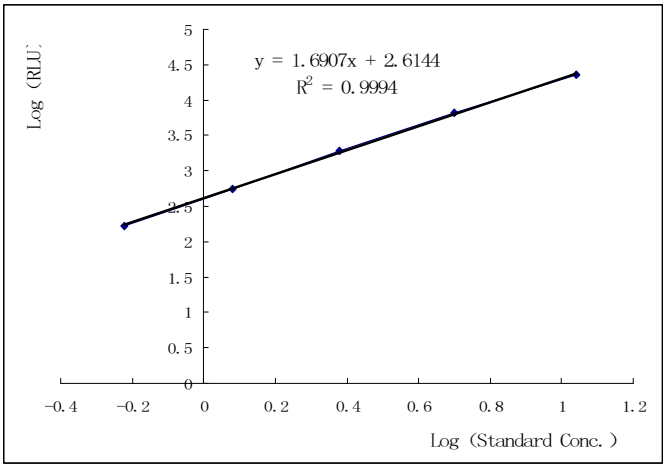
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. Plot the $\log_{10}$RLU for each reference standard against the common logarithm of corresponding concentration of C-P in ng/ml on logarithmic graph paper, with RLU values on the Y-axis and and concentration on the X- axis.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

C-P (ng/ml)	RLU
0.6	168.7
1.2	549.4
2.4	1917.0
5	6537.4
11	22427.7



EXPECTED VALUES

Each laboratory must establish its own normal range based on patient population. The normal range is between 0.4ng/ml and 5.7ng/ml, which were determined by testing 200 fasting sera samples.

PERFORMANCE

A. Sensitivity

The lower detection limit is calculated from the standard curve by identifying the concentration corresponding to the mean RLU of standard diluent (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Autobio C-P CLIA kit is estimated to be not higher than 0.3ng/ml.

B. Specificity

No interference was detected with the performance of Autobio C-P CLIA upon addition of massive amounts of the following substances to a human serum pool.

Interferents	Concentration
Proinsulin	1000pmol/l

C. Precision

a. Intra-assay Precision

Intra-assay precision was determined by assaying 20 replicates of each control sera.

Serum	Number	Mean	SD	CV (%)
Low titer	20	2.01	0.09	4.37
High titer	20	4.66	0.25	5.48

b. Inter-assay Precision

Inter-assay precision was determined by assaying duplicates of each control sera in 10 separate runs.

Serum	Number	Mean	SD	CV (%)
Low titer	10	1.78	0.13	7.11
High titer	10	4.06	0.26	6.53

D. High Dose Hook Effect

In this C-P assay, patient samples spiked to C-P levels up to 21ng/ml do not demonstrate aparadoxical decrease in the RLUs (high dose hook effect).

E. Accuracy

For samples in the range of 0.3ng/ml to 11ng/ml, the correlation between the Autobio C-P CLIA kit and Beckman access 2® C-P assay,are described by the equation:

Reference	Number of Specimens	Least Square Regression Analysis	Correlation Coefficient
Beckman access 2®	180	y = 0.9385x + 1.0452	0.9688

LIMITATIONS

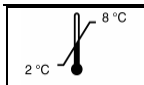
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert and with adherence to good laboratory practice.
2. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

LOT	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
IVD	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**

	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES

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For order and inquires, please contact

AUTOBIO DIAGNOSTICS CO., LTD.
ADD: No.87 Jingbei Yi Road, National Eco & Tech Development Area,
Zhengzhou , China 450016
Tel: +86-371-67985313 Fax: +86-371-67985804
Web: www.autobio.com.cn