

B) External Evaluation:

Qualisa™ C-Peptide ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Qualisa™ C-Peptide** ELISA has demonstrated 100% correlation with the reference method.

*Data file: Zephyr Biomedical (A Division of Tulip Diagnostics (P) Ltd).

IMPORTANT NOTE

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.












LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

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SYMBOL KEYS

 Temperature Limitation	 Consult Instructions for use	 Date of Manufacture	 Batch Number / Lot Number
 Manufacturer	 In vitro Diagnostic Medical Device	 This side up	 Contains sufficient for <n> tests
 Use by	 Catalogue Number	 Do not reuse	



Manufactured by:

Zephyr Biomedicals

A Division of Tulip Diagnostics (P) Ltd.

M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA.

Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.



Enzyme linked Immunosorbent Assay (ELISA) for Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Qualisa™ C-Peptide test is intended for the Quantitative Determination of Circulating C-Peptide Concentrations in Human Serum. For In Vitro Diagnostic Use Only.

INTRODUCTION

Diabetes is one of the leading causes of disability and death in the U.S. It affects an estimated 16 million Americans, about one third of them do not even know they have the disease. The causes of diabetes are not precisely known, but both genetic and environmental factors plays significant roles. The disease is marked by deficiencies in the body's ability to produce and properly use insulin. The most common forms of diabetes are type 1, in which the body's ability to produce insulin is destroyed, and type 2, in which the body is resistant to insulin even though some amount of insulin may be produced.

In-vitro determination of insulin and *C-Peptide* levels help in the differential diagnosis of liver disease, acromegaly, Cushing's syndrome, familial glucose intolerance, insulinoma, renal failure, ingestion of accidental oral hypoglycemic drugs or insulin induced factitious hypoglycemia. Both insulin and *C-Peptide* are produced by enzymatic cleavage of proinsulin. Proinsulin is stored in the secretory granules of pancreatic β -cells and is split into a 31 amino acid connecting peptide (*C-Peptide*; MW 3600) and insulin (MW 6000). *C-Peptide* is devoid of any biological activity but appears to be necessary to maintain the structural integrity of insulin. Although insulin and *C-Peptide* are secreted into portal circulation in equimolar concentrations, fasting levels of *C-Peptide* are 5-10 fold higher than those of insulin owing to the longer half-life of *C-Peptide*. The liver does not extract *C-Peptide* however; it is removed from the circulation by degradation in the kidneys with a fraction passing out unchanged in urine. Hence urine *C-Peptide* levels correlate well with fasting *C-Peptide* levels in serum. The glucagon stimulated *C-Peptide* determination is often used for differential diagnosis of insulin-dependent from non-insulin-dependent diabetic patients.

PRINCIPLE

The **Qualisa™ C-Peptide** Quantitative Test Kit is based on a solid phase enzyme-linked immunosorbent assay. The assay system utilizes one anti-C-Peptide antibody for solid phase (microtiter wells) immobilization and another anti-C-Peptide antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The standards and test specimen (serum) are added to the C-Peptide antibody coated microtiter wells. Then anti-C-Peptide antibody labeled with horseradish peroxidase (conjugate) is added. If human C-Peptide is present in the specimen, it will combine with the antibody on the well and the enzyme conjugate resulting in the C-Peptide molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed & bound enzyme is detected by adding substrate. The reaction is stopped after specified time with stop solution and absorbance is determined for each well using an ELISA reader. The concentration of C-Peptide is directly proportional to the color intensity of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti-C-Peptide antibody.
- Enzyme Conjugate. Ready to use.
- TMB Substrate. Ready to use.
- Stop Solution. Ready to use.
- C-Peptide Standard set of 6 standards labeled as A to F in Lyophilized form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided

- Precision pipettes: 10-100 μ l, 20-200 μ l, 100-1000 μ l
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Qualisa™ C-Peptide** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated Microwells should be used within one month upon opening the pouch provided that once opened, the pouch must

be resealed to protect from moisture. If the colour of the desiccant has changed from blue to white at the time of opening the pouch, another coated Microwells pouch should be used.

- Diluted Wash Buffer is stable upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

- Only fasting morning fresh serum samples to be used for testing.
- Samples should be assayed as fresh as possible. If the samples cannot be assayed on the same day when collected, then the samples may be stored at -20°C for up to 30 days.
- Collect Blood specimen by venipuncture according to standard procedure.
- Only serum should be used.
- Avoid grossly hemolytic, lipemic or turbid samples.
- Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
- For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
- Do not heat/inactivate before use.
- Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
- Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

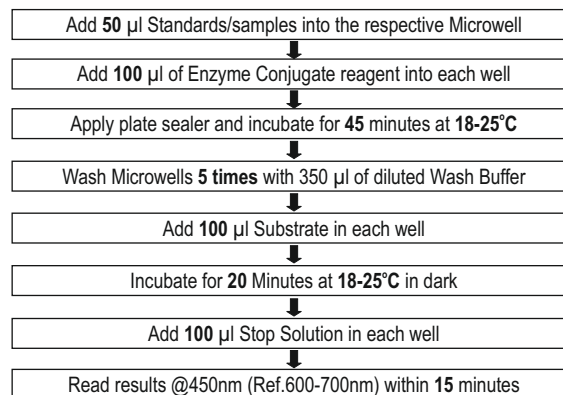
- Bring all reagents and specimen to room temperature before use.
- Do not pipette any material by mouth.
- Do not eat, drink or smoke in the area where testing is done.
- Use protective clothing and wear gloves when handling samples.
- Use absorbent sheet to cover the working area.
- Immediately clean up any spills with sodium hypochlorite.
- All specimens and standards should be considered potentially infectious and discarded appropriately.
- Neutralize acid containing waste before adding hypochlorite.
- Do not use kit after the expiry date.
- Do not mix components of one kit with another.
- Always use new tip for each specimen and reagent.
- Do not allow liquid from one well to mix with other wells.
- Do not let the strips dry in between the steps.

REAGENT PREPARATION

- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

TEST PROCEDURE

- Secure the desired number of coated wells in the holder. Dispense 50 µl of standards and serums into the appropriate wells.
- Dispense 100 µl of Enzyme Conjugate reagent into each well. Incubate at room temperature (18-25°C) for 45 mins.
- Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
- Dispense 100 µl of TMB Substrate into each well. Incubate at room temperature (18-25°C), in the dark, for 20 minutes.
- Stop the reaction by adding 100 µl of Stop Solution to each well. Gently mix for 10 seconds until the blue color completely changes to yellow.
- Read the optical density at 450/630 nm with a microtiter plate reader within 15 minutes.



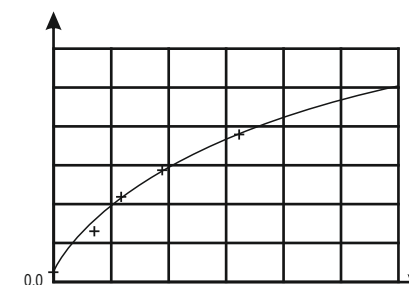
CALCULATION OF RESULTS

Construct a standard curve by plotting the absorbance obtained from each reference standard against its concentration in ng/ml on the graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the absorbance values for each specimen to determine the corresponding concentration of C-Peptide in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical standard run with optical density reading at 450nm (ref 600 – 700nm) shown in the Y axis against C-Peptide concentrations shown in the X axis.

C-Peptide (ng/ml)	Absorbance (450nm)
A	0.002
B	0.031
C	0.091
D	0.224
E	0.670
F	1.544



This Standard curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own standard curve and data.

Expected Ranges of values

C-Peptide values are consistently higher in plasma than in serum; thus, serum is preferred. Compared with fasting values in non-obese non-diabetic individuals, C-Peptide levels are higher in obese non-diabetic subjects and lower in trained athletes. Each laboratory is advised to establish its own ranges for normal and abnormal populations. These ranges are always dependent upon locale, population, laboratory, technique and specificity of the method.

Adult (Normal) 0.7 – 1.9 ng/ml

The minimum detectable concentration of C-Peptide by this assay is estimated to be 0.03 ng/ml

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

- Accuracy: In an internal Study **Qualisa™ C-Peptide** was evaluated against commercially available licensed kit with 90 random clinical samples and **Qualisa™ C-Peptide** has demonstrated 100% clinical correlation with the commercially available licensed kit.
- Precision: **Qualisa™ C-Peptide** was evaluated with licensed external Quality controls for Precision Studies & following is the data:

Controls	No. of testing's	Mean Control values with Qualisa™ C-Peptide	Coefficient of Variation (CV)
Level 1	10	0.462	8.52
Level 2	10	3.96	7.25
Level 3	10	5.32	6.47