



UniQ™

ICTP<sub>EIA</sub>

**C-terminal telopeptide of type I collagen  
Enzymeimmunoassay kit  
Cat. No. 05892**

**Instructions for use**

**April 2004**

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**ISO9001  
CERTIFIED**

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## Explanation of symbols used on labelling


**IVD** For in vitro diagnostic use

**REF** Catalogue number

**LOT** Batch code

 Use by

 Storage temperature

 Consult instructions for use

**CONJ** | **ENZ** Enzyme conjugate

**CAL** | **0** Calibrator concentration

**CONTROL** Control

**Ab** | **ICTP** Antiserum

**BUF** | **WASH** | **12.5X** Wash concentrate

**SOLN** | **STOP** Stopping solution

**SUBS** | **TMB** Substrate

## **INTENDED USE**

Orion Diagnostica UniQ ICTP EIA is a quantitative enzymeimmunoassay designed for *in vitro* measurement of carboxyterminal cross-linked telopeptide of type I collagen concentration in human serum.

## **CLINICAL IMPORTANCE OF ICTP**

Type I collagen is the most abundant collagen type in the body and the only collagen type found in mineralised bone, where it accounts for more than 90 % of the organic matrix. In addition, type I collagen is found in loose connective tissues together with other collagen types such as types III, V and VI. Also in these locations the proportion of type I collagen is the largest.

Type I collagen in tissues is present in fibres, the structure of which shows some variation according to tissue. In bone the type I collagen molecules are cross-linked via three residues of hydroxylysine, lysine or their derivatives which form a fluorescent, cyclic pyridinoline-structure and non-fluorescent unknown structures linking three different collagen polypeptide chains together. In loose connective tissues, such as skin, the major mature cross-link of type I collagen is non-fluorescent and contains histidine as one of the amino acid residues.

ICTP is the carboxyterminal telopeptide region of type I collagen, joined via trivalent cross-links and liberated during the degradation of mature type I collagen. This peptide is found in an immunochemically intact form in blood, where it seems to be derived from bone resorption and degradation of loose connective tissues. It has recently been shown, that the ICTP antigen is produced through the action of e.g. matrix metalloproteinases, which are enzymes involved in tissue destruction in various pathological conditions.

Increased serum concentrations of ICTP are hence seen in conditions associated with increased lysis of bone, such as multiple myeloma, osteolytic metastases, rheumatoid arthritis and e.g. immobilisation. As ICTP is not produced through physiological, Cathepsin-K mediated bone resorption, its concentration is less affected by e.g. menopause.

## **PRINCIPLES OF THE TEST**

The Orion Diagnostica UniQ ICTP EIA kit is based on the competitive immunoassay technique. A known amount of peroxidase labelled ICTP and an unknown amount of unlabelled ICTP in the sample compete for the limited number of high affinity binding sites of the primary antibody. A secondary antibody, directed against the primary and coated to the wells, binds the antibody-antigen complex, which enables convenient separation of bound and free antigen. After washing away the free antigen, the amount of labelled ICTP in the well is inversely proportional to the amount of ICTP in the sample. The amount of labelled ICTP is measured by incubation with a substrate that produces a coloured end product. The concentrations in unknown samples are obtained from a calibration curve.

# REAGENTS

## Materials provided

REAGENTS	QUANTITY	STORAGE
<b>ICTP EIA MICROTITRE PLATE</b> 96 wells, coated with goat anti-rabbit antibodies.	12x8 wells	2...8 °C in the original package until expiry date. Close the package tightly after use.
<b>ICTP EIA ENZYME CONJUGATE</b> Ready to use peroxidase labelled ICTP. Red colour additive.	1 vial 7 ml	2...8 °C until expiry date
<b>ICTP EIA ANTISERUM</b> Ready to use rabbit antiserum. Blue colour additive.	1 vial 7 ml	2...8 °C until expiry date
<b>ICTP EIA CALIBRATORS</b> Lyophilised 0, 1.0, 2.5, 5.0, 10, 25 and 50 µg/l in phosphate buffer. <i>Calibrated against an in-house master calibrator set.</i>	7 vials  Reconstitute with 0.5 ml of distilled water	2...8 °C until expiry date  2...8 °C 7 weeks after reconstitution.
<b>ICTP EIA CONTROLS 1&amp;2</b> Lyophilised, in human serum. Expected values are indicated on a separate sheet.	2 vials  Reconstitute with 0.5 ml of distilled water	2...8 °C until expiry date  After reconstitution aliquot and store at -20 °C. Avoid repeated freezing and thawing.
<b>ICTP EIA WASH CONCENTRATE</b> 12.5 x concentrated	1 bottle 80 ml Dilute to 1000 ml with distilled water	2...8 °C until expiry date  2...25 °C 7 weeks after dilution.
<b>ICTP EIA SUBSTRATE</b> Ready to use 3,3',5,5'-Tetramethylbenzidine in aqueous buffer.	1 bottle  17 ml	2...8 °C until expiry date
<b>ICTP EIA STOPPING SOLUTION:</b> Ready to use 0.5 M H <sub>2</sub> SO <sub>4</sub>	1 bottle  13 ml	2...8 °C until the expiry date

## **Warnings and precautions**

**CAUTION 1: For *in vitro* diagnostic use.** Not for internal or external use in humans or animals. Do not mix the components of separate kit lot numbers. Use only the components belonging to the same kit lot. Do not use reagents after the expiration date stated on each reagent container's label.

Do not pipette by mouth. Do not eat, drink or smoke while handling the reagents. Wear disposable gloves and suitable protective clothing. Avoid contact with skin and eyes. Wash hands thoroughly after handling the reagents. In case of accidental spills wash with large amount of water.

Disposal of all waste should be in accordance with local regulations.

**CAUTION 2:** All patient specimens should be handled as **potentially infectious**. Source material of human blood from which the controls for the kit were derived was found non-reactive to HBsAg and negative for HIV1, HIV2 and HCV antibodies when tested with licensed reagents. However, as no known test can provide assurance that these materials are not infectious, they should be handled as if capable of transmitting infectious diseases.

**CAUTION 3:** The Stopping Solution is a 0.5 M sulphuric acid solution. It is corrosive. Handle and dispose with care.

## **INSTRUMENTS**

Plate shaker, plate washer and photometric plate reader (measuring absorbances at 450 nm) are needed. It is recommended to calibrate the instruments systematically. Consult the operations manual supplied by the instrument manufacturers for details of installation, use, maintenance and calibration. Shaking speed at least 600 rpm.

## **SPECIMEN HANDLING AND STORAGE**

Although serum samples are recommended, the kit may also be used with EDTA-plasma samples. Do not use heparin or citrate plasma samples.

Serum samples may be stored for up to 5 days at 2...8 °C, and for longer periods at least at -20 °C. Repeated freezing and thawing should be avoided.

## PROCEDURE

### Materials required but not provided

PIPETTES (50  $\mu$ l, 100  $\mu$ l, 500  $\mu$ l), MICROTITRE PLATE SHAKER, MICROTITRE STRIP WASHER, PHOTOMETRIC PLATE READER, ABSORBENT PAPER, DISTILLED WATER

### Reconstitution of reagents

**Calibrators and controls.** Allow the vials to reach equilibrium at room temperature (18...25 °C) before opening. **Reconstitute** by adding 500  $\mu$ l of distilled water to all vials. Cap and mix well by gentle swirling or inversion to avoid foaming. Allow to stand for 30 minutes before use.

**Wash concentrate.** Dilute the wash concentrate to 1000 ml (80 ml + 920 ml) with distilled water.

### Details of the procedure

1. **Bring** all reagents, controls and patient samples to room temperature (18...25 °C) at least 30 minutes before use.
2. **Remove** excess strips from the plate frame, **return** them to the pouch and close tightly.
3. **Pipette** 50  $\mu$ l of calibrator, control and patient sample in duplicate into appropriate microtitre wells. Reserve two wells for the substrate blank.
4. **Pipette** 50  $\mu$ l of ICTP enzyme conjugate (red) into all wells except blanks.
5. **Pipette** 50  $\mu$ l of ICTP antiserum (blue) into all wells except blanks. The antiserum must be applied to all wells within 3 minutes. The use of an electronic dispenser or multichannel pipette is recommended.
6. **Incubate** on a plate shaker at 18...25°C for 2 hours. Use a shaking **speed** of **600-1000 rpm**.
7. **Wash** the strips 4 times with the wash solution on a plate washer. Use an appropriate volume of the wash solution (300-500  $\mu$ l/well is recommended) and program for flat-bottom plates. Remove any remaining moisture by tapping the strips firmly against absorbent paper. Press the long sides of the holder firmly, so that the strips do not drop off. **Note: Proper washing is crucial for the assay performance.**
8. **Pipette** 100  $\mu$ l of ICTP substrate into all wells.
9. **Incubate** on a plate shaker at 18...25 °C for 30 minutes.
10. **Stop** the enzyme reaction by adding 100  $\mu$ l of stopping solution into all wells. **Shake** for 15-30 seconds to mix the reagents.
11. **Read** the absorbances of all wells at 450 nm on a plate reader within 10 minutes.

## LIMITATIONS OF PROCEDURE

There is a diurnal variation of ICTP the values being higher at night. Renal insufficiency, glomerular filtration rate of 50 ml/min/1.73 m<sup>2</sup> or less, leads to elevated ICTP concentrations in blood. The liver does not seem to be involved in the metabolism of the serum ICTP antigen. Samples containing heterophilic antibodies may cause erroneous results.

As a basis for diagnostic and therapeutic decisions the results of any single diagnostic test must be backed up with other clinically relevant data.

## QUALITY ASSURANCE

An internal quality assurance program is recommended for all clinical laboratories, thus the analysis of control sera in both the lower and upper portions of the calibration curve is suggested for monitoring the performance of the procedure. It is recommended that each laboratory establish their own acceptable ranges for the controls used in that laboratory. The mean of the control should fall within the acceptable range stated by the manufacturer. It is advised to use at least two separate kit lot numbers for the control value assignment.

## CALCULATION OF RESULTS

For automatic result processing spline function curve fitting is recommended. Results can also be produced manually on semi-log graph paper.

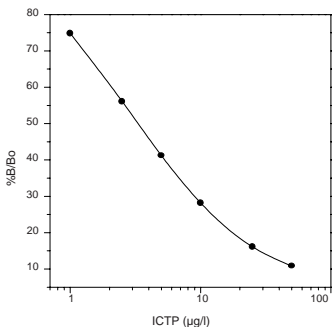
**Calculate** the mean absorbances for all calibrators, samples and controls.

**Calculate** the %B/B<sub>0</sub> from:

$$\%B/B_0 = \frac{(\text{calibrator or sample absorbance} - \text{blank}) \times 100}{(0\text{-calibrator absorbance} - \text{blank})}$$

**Draw** a calibration curve on semi-log graph paper with %B/B<sub>0</sub> values on the ordinate and the ICTP concentrations (µg/l) of the calibrators on the abscissa.

Read the ICTP concentrations of the unknowns from the calibration curve.



Well	mean absorbance	absorbance-blank	%B/B <sub>0</sub>	ICTP (µg/l)
Blank	0.072			
Cal 0	1.857	1.785		
Cal 1.0	1.407	1.335	74.8	
Cal 2.5	1.073	1.001	56.1	
Cal 5.0	0.808	0.736	41.2	
Cal 10	0.575	0.503	28.2	
Cal 25	0.360	0.288	16.1	
Cal 50	0.264	0.192	10.8	
Unkn 1	0.818	0.746	41.8	4.8
Unkn 2	0.484	0.412	23.1	13.9

**Figure 1**

Typical calibration curve. Example.

**Table 1**

Calculation of results using typical data. Example.

## REFERENCE LIMITS

Due to ethnic, dietary and age variations, the reference limits given may not apply to all populations. Therefore each laboratory should establish its own representative reference limits.

ICTP values of apparently healthy adults (19-74 years) were measured with the ICTP EIA kit. The non-parametric method recommended by IFCC was used to calculate the reference intervals for the upper and lower limits.

**Table 2** Serum ICTP reference limits

	Female	Male
Number of subjects	146	127
Mean	2.6 µg/l	2.7 µg/l
Reference interval*	<b>1.6 - 4.2 µg/l</b>	<b>1.5 - 4.3 µg/l</b>
0.90-confidence intervals		
for lower reference limit	1.5 - 1.8 µg/l	1.2 - 1.6 µg/l
for upper reference limit	3.8 - 5.2 µg/l	3.9 - 5.3 µg/l

\* Reference interval = 0.025 & 0.975 fractiles

## PERFORMANCE CHARACTERISTICS

### Measurement range

1.0-50 µg/l.

### Detection limit

Approximately 0.3 µg/l, defined as twice the standard deviation of the 0-binding value.

### Dilution

Samples with high ICTP concentrations may be diluted using saline or the 0-calibrator of the kit.

### Recovery

Known amounts of ICTP were added to five patient serum samples containing 3.4 – 6.2 µg/l of ICTP. Recoveries were in the range of 88 - 95 % with a mean value of 93 %.

### Precision

Intra- and inter-assay variations were determined using native and spiked serum pools containing different concentrations of the ICTP antigen.

**Table 4** Intra-assay / Inter-assay precision

Intra-assay precision of 10 replicates			Inter-assay precision of 10 duplicate determinations		
Sample	Mean (µg/l)	CV (%)	Sample	Mean (µg/l)	CV (%)
1	2.9	11.3	6	3.2	6.4
2	4.7	13.2	7	4.8	7.6
3	5.6	8.1	8	7.1	7.5
4	13.7	8.9	9	13.1	9.8
5	26.4	7.6	10	28.2	6.4

### Interfering substances

Serum bilirubin concentration < 340 µmol/l does not interfere. Serum haemoglobin concentration up to 5 g/l does not interfere. Triglycerides up to 30 g/l do not interfere.

### Specificity

The antigenic determinant of the antibody is within the two hydrophobic phenylalanine-rich sequences of the trivalently cross-linked ICTP. A trivalent cross-link is necessary, the divalently cross-linked and monomeric peptides containing only one phenylalanine-rich sequence show poor immunoreaction.

The ICTP EIA antiserum does not cross-react with antigens used in other UniQ Collagen assays.

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UniQ™

ICTP<sub>EIA</sub>

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Enzymeimmunoassay kit  
Cat. No. 05892**

**Kit Contents**

- (Kit sufficient for 96 tests)
- ICTP EIA microtitre plate
- ICTP EIA enzyme conjugate
- ICTP EIA antiserum
- ICTP EIA calibrators
- ICTP EIA controls
- ICTP EIA wash concentrate
- ICTP EIA substrate
- ICTP EIA stopping solution

**Assay procedure - summary**  
*(all volumes given in µl)*

	Blank	Calibrator	Control/ unknown
Pipette sample		50	50
Pipette enzyme conjugate (red)		50	50
Pipette antiserum (blue)		50	50
Incubate on plate shaker for 2h at 18...25 °C	x	x	x
Wash 4 times	x	x	x
Pipette substrate	100	100	100
Incubate on plate shaker for 30 min at 18...25 °C	x	x	x
Pipette stopping solution	100	100	100
Shake briefly to mix the reagents	x	x	x
Read absorbances at 450 nm	x	x	x

US Patent No. 5,538,853

EP Patent No. 505210

JP Patent No. 2886728