# Alpha CrossLaps® (CTX-I) EIA

Instructions For Use

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| **Product Name** | Alpha CrossLaps® (CTX-I) EIA | REF | AC-04F1R |
| **Abbreviated Product Name** | Alpha CTX-I EIA | | |

## 1. Intended Use

**Not for use in clinical or diagnostic procedures.**

The Alpha CrossLaps® (CTX-I) EIA is an enzyme immunological test for the quantification of degradation products of C-terminal telopeptides of Type I collagen in human urine. Results are to be used for performance evaluation / investigational / research use only.

## 2. Summary and Explanation

Type I collagen accounts for more than 90% of the organic matrix of bone and is synthesized primarily in bone. During renewal of the skeleton, Type I collagen is degraded, and small peptide fragments are excreted into the urine. These fragments can be measured by Alpha CTX-I EIA. The measurements of the specific degradation products of Type I collagen (Alpha CrossLaps) in human urine have been reported as useful assessment of bone resorption in Paget's disease[1] and for detection of bone metastases in prostate[2, 5] and breast cancer[3-6].

## 3. Method Description

The Alpha CrossLaps® (CTX-I) EIA assay is based on one highly specific monoclonal antibody against the amino acid sequence of EKAHDGGR. In order to obtain a specific signal in the Alpha CTX-I EIA, two chains of EKAHDGGR must be cross linked.

Calibrators, Controls, or unknown samples are pipetted into the appropriate microtiter wells coated with streptavidin, followed by application of a mixture of biotinylated antibody and peroxidase-conjugated antibody. Then, a complex between ALPHA CrossLaps antigens, biotinylated antibody and peroxidase-conjugated antibody is generated, and this complex binds to the streptavidin surface via the biotinylated antibody. Following the one step incubation at 2-8°C, the wells are emptied and washed. After the washing step, the wells are incubated with a chromogenic substrate. The reaction is stopped, and the absorbance is measured.

## 4. Warnings and Precautions

The Alpha CTX-I EIA is for performance evaluation / investigational / research use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions For Use (IFU). Immunodiagnostic Systems Limited (IDS) will not be held responsible for any loss or damage (except as required by statute), howsoever caused, arising out of non-compliance with the instructions provided.

**CAUTION:** This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent. Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

**Human materials**

Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled according to Biosafety Level 2.

**Reagents containing Sodium Azide**

Some reagents in this kit contain sodium azide ($NaN_3$) <0.1 % (w/w) which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.
Classification under CLP:
EUH208

Hazard statements:
EUH208 Contains a mixture of: 5-chloro-2-methyl-2h-isothiazol-3-one [ec no 247-500-7] and 2-methyl-2h-isothiazol-3-one [ec no 220-239-6]. May produce an allergic reaction.

Precautionary statements:
N/A

## 5. Shelf Life And Storage Of Reagents

This kit is stable until the expiry date printed on the box if stored as specified. Upon receipt, store all reagents at 2-8°C.
Do not use any kit component beyond their expiry date.

Do not use kit components beyond the expiry date and do not mix reagents from different lots.

Indications of possible deterioration of kit reagents include:

- The presence of abnormal particulate matter in any of the reagents.
- A decrease in the maximum binding.
- A high non-specific binding.
- A shift in the slope of the curve from its normal position.

## 6. Sample Collection and Storage

For optimal results it is recommended to use urine from second morning void from fasting individials. For monitoring purpose, follow up samples should be collected under same conditions as the baseline sample.
Urine samples are stable for 7 days at 4°C. For longer storage, the urine samples should be stored frozen (< -18°C). Prior to use, urine specimens should be shaken and sedimentation allowed for a minimum of 30 minutes.
Specimens obviously contaminated with whole blood may interfere with assay performance. These specimens should be discarded and a new specimen collected.

**Note:**

- Samples containing particulate matter must be centrifuged before performing the assay.
- Samples displaying microbial contamination should not be assayed with the kit.
- Before performing assays, make sure that samples, calibrators and controls are at room temperature (18 - 22 °C).
- Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on appropriate practices, please refer to the CLSI GP44-A4, “Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests”.

## 7. Materials

**Materials Provided**

**MICROPLAT** **Streptavidin coated microtiter plate**
Microwell strips (12x8 wells) pre-coated with streptavidin. Supplied in a plastic frame.

**CAL0** **Calibrator**
Ready to use buffered solution with protein stabiliser, detergent and preservative; 1 vial, 12.0 mL

**CAL 1 -5** **Calibrators**
Ready to use buffered solution containing human CrossLaps® antigen with protein stabilisers, detergent and preservative; 1 each of 5 concentration levels, 0.4 mL per vial
The exact value of each standard is printed on the QC report.

**CTRL 1 – 2** **Controls**
Ready to use buffered solution containing human CrossLaps® antigen with protein stabilisers, detergent and preservative; 1 each of 2 concentration levels, 0.4 mL per vial
The established ranges for the controls are printed on the QC report.

**AB BIOTIN** **Biotinylated Antibody**
Concentrated solution of biotinylated monoclonal (murine) anti-CTX-I antibody prepared in a buffered solution with protein stabiliser, detergent and preservative; 1 vial, 0.2 mL

**ENZYMCONJ** **Peroxidase Conjugated Antibody**
Concentrated peroxidase conjugated monoclonal antibody specific for CTX-I; provided in a buffered solution with protein stabiliser; 1 vial, 0.25 mL.

**BUF** **Incubation Buffer**
Ready to use buffered solution containing protein stabiliser, detergents and preservative; 1 vial, 15.0 mL

**SUBS TMB** **Substrate Solution**
Ready to use tetramethylbenzidine (TMB) substrate in an acidic buffer; 1 vial, 12.0 mL
Please note that the chromogenic substrate might appear slightly blueish.

**$H_2SO_4$** **Stopping Solution**
Ready to use solution of 0.18 mol/L sulphuric acid; 1 vial, 12.0 mL

**WASHBUF 50x** **Washing Buffer**
Concentrated washing buffer with detergent and preservative; 1 vial, 20.0 mL

**Adhesive Plate Sealer** Adhesive film for covering wells during incubation.
**Documentation** Instructions for Use and QC report.

**Materials required — not supplied**
- Containers for preparing the Antibody Solution and the Washing Solution
- Precision pipetting devices to deliver 25 – 200 µL
- Distilled water
- Precision 8 or 12 channel multipipette to deliver 100 µL to 300 µL
- Vortex mixer
- Automatic microplate washer (optional)
- Photometric microplate reader and data analysis equipment

## 8. Preparation Of Reagents

Allow all reagents to come to room temperature (18 - 22°C) for a minimum of 60 minutes before use. Do not interchange kit components from different lots.

**Antibody Solution**
**Prepare the Antibody Solution immediately before use**. Mix the Biotinylated Antibody **AB BIOTIN**, Peroxidase Conjugated Antibody **ENZYMCONJ** and Incubation Buffer **BUF** in the volumetric ratio 1 + 1 + 100 in an empty container. Mix carefully and avoid formation of foam.
**Prepare a fresh solution before each run of the assay.**

**Wash buffer preparation**
Prepare by adding 1-part Wash Concentrate **WASHBUF 50x** to 50-parts distilled water.

All other reagents are supplied ready for use.

**N.B**. To avoid potential microbial and / or chemical contamination, unused reagents should never be returned into the original vials.

## 9. Assay Procedure

Prepare reagents as described in § 8. Preparation of Reagents. Mix all reagents and samples before use (avoid formation of foam).

**NOTE:** To ensure consistent results between runs, between operators, and to minimise any drift effect; strictly adhere to the following procedure:
a. Bring all reagents to room temperature (18 – 22 ºC) prior to use – this will take approximately 60 minutes.
b. Seal the plate during incubations using the plate sealers which are supplied with the assay kit.
c. Do not stack plates during incubation in order to ensure a consistent temperature for all plates.
d. Do not under or over-fill the assay wells during the washing steps.
e. Add reagents in the same sequence each time to reduce time deviation between reactions

Do not pipette directly from the vial containing TMB substrate. The required volume should first be transferred to a clean container. Solution remaining in the container should be discarded following use and NOT returned to the stock vial **SUBS TMB**.

Determine the number of strips needed for the assay; it is recommended to test all samples in duplicate. In addition, for each run a total of 16 wells are needed for the standards and controls. Place the appropriate number of strips in the plastic frame. Store any unused strips in the tightly closed foil bag with desiccant capsules.

1. Dilute **Control** **CTRL 1 - 2**, supplied with the kit and urine samples 1+7 in **Calibrator 0** **CAL 0** prior to testing (e.g. 25+175 µL).
2. Pipette 25 µL of **Calibrators** **CAL 0 – 5**, **Controls** **CTRL 1 - 2** or unknown samples into appropriate wells followed by 100 µL of the **Antibody Solution.**
3. Cover the immunostrips with sealing tape and incubate for 60±5 minutes at 2-8°C without shaking.
4. Wash all wells 5 times with wash buffer

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   | Automatic plate wash | Set plate washer to dispense 300 µL of wash solution per well<br>Fill and aspirate for 5 cycles |
   | Manual wash | Decant the contents of the wells by inverting sharply<br>Pipette 300 µL of wash solution into each well, decant and repeat 5 times<br>Remove excess wash buffer by tapping firmly on absorbent tissue before proceeding |

   Make sure wells are completely emptied after each manual or automatic washing cycle.
   Proceed immediately to the next step at the end of washing.
5. Pipette 100 µL of the Substrate Solution **SUBS TMB** into each well and incubate for 15±2 minutes at room temperature (18-22°C) in the dark. Use sealing tape.
   **NOTE:** do not pipette directly from the vial containing TMB substrate. The required volume should first be transferred to a clean container. Solution remaining in the container should be discarded following use and NOT returned to the stock vial **SUBS TMB**.
6. Pipette 100 µL of the **Stopping Solution** **$H_2SO4$** into each well.
7. Measure the absorbance at 450 nm with 650 nm as reference within two hours.

**N.B**. Microplate readers measure vertically; when pipetting, do not touch the bottom of the wells

**Automated Platforms**
The Alpha CrossLaps® (CTX-I) EIA kit was designed and developed to be performed manually using the protocol described above. The protocol is not necessarily applicable to automated platforms.

If automated platforms are used it is the responsibility of the user to ensure the kit has been appropriately tested. To improve the performance of the kit on automated platforms, it is recommended to increase the number of wash cycles at each wash step.

## 10. Calculation of Results

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic curve fit should be used.

Alternatively, calculate the mean of the duplicate absorbance determinations. Construct a standard curve on graph paper by plotting the mean absorbances of the six Calibrators on y-axis against the corresponding CrossLaps concentrations on x-axis. Determine the CrossLaps concentration of the controls and each patient sample by interpolation.

If the absorbance of a pre-diluted sample is above **Calibrator 5,** the sample should be diluted in **Calibrator 0** and re-analysed.

For each urine sample the Alpha CrossLaps concentration (ng/mL) and the creatinine concentration (mM= mmol/L) should be determined using an enzymatic colometric method for clinical chemistry analyser.

The following equation corrects the Alpha CrossLaps concentration for variation in urine concentration:

Corr. Alpha CrossLaps Value (µg/mmol) = Alpha CrossLaps (ng/mL) ÷ Creatinine (mmol/L)

## 11. Quality Control

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples and the results analysed with appropriate statistical methods.

The two kit controls provided in the kit are intended to assist in assessing the validity of results obtained with each assay plate.

IDS recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The trending will assist in the identification of assays which give control values significantly different from their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[7].

## 12. Measurement Range

Detection limit: 0.80 ng/mL Alpha CrossLaps

This is the concentration corresponding to three standard deviations above the mean of 21 determinations of the blank (Standard 0) multiplied by the dilution factor 8.

## 13. Limitations of Use

- Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

## 14. Symbols used

Catalogue Number

Manufacturer

## 15. REFERENCES


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## Assay Procedure