

Product Name	Serum CrossLaps [®] (CTX-I) ELISA	REF AC-02F1R
Abbreviated Product Name	Serum CTX-I ELISA	

INTENDED USE

Not for use in clinical or diagnostic procedures.

The Serum CrossLaps[®] (CTX-I) ELISA is an enzyme immunological test for the quantification of degradation products of C-terminal telopeptides of Type I collagen in human serum and plasma. Results are to be used for performance evaluation / investigational / research use only.

Summary and explanation of the test

Type I collagen accounts for more than 90% of the organic matrix of bone and is synthesized primarily in bone (1). During renewal of the skeleton, Type I collagen is degraded, and small peptide fragments are excreted into the bloodstream. These fragments can be measured by Serum CrossLaps[®] (CTX-I) ELISA. The measurements of the specific degradation products of Type I collagen in both urine (2) and serum (3) by a competitive CrossLaps have been reported.

Principle of the procedure

The Serum CrossLaps[®] (CTX-I) ELISA is based on two highly specific monoclonal antibodies against the amino acid sequence of EKAHD- β -GGR, where the aspartic acid residue (D) is β -isomerized. In order to obtain a specific signal in the Serum CrossLaps[®] (CTX-I) ELISA, two chains of EKAHD- β -GGR must be cross linked. Standards, control, or unknown serum samples are pipetted into the appropriate microtitre wells coated with streptavidin, followed by application of a mixture of a biotinylated antibody and a peroxidase conjugated antibody. Then, a complex between 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 room temperature, the wells are emptied and washed. A chromogenic substrate is added and the colour reaction is stopped with sulfuric acid. Finally, the absorbance is measured.

PRECAUTIONS

The following precautions should be observed in the laboratory:

- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled
- Do not pipette by mouth.
- Wear gloves when handling immunodiagnostic materials and wash hands thoroughly afterwards
- Cover working area with disposable absorbent paper

WARNINGS

The Serum CrossLaps[®] (CTX-I) ELISA 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.

HAMA interference

Some individuals have antibodies to mouse immunoglobulins (HAMA), which can cause interference in immunoassays that employ murine monoclonal antibodies, such as Serum CrossLaps. In rare cases, the content of HAMA exceeds the capacity of the blocking agent incorporated into Serum CrossLaps leading to a false-positive test result. Therefore, Serum CrossLaps values should be used only in conjunction with information available from the clinical evaluation of the patient.

Storage

Store the Serum CrossLaps® (CTX-I) ELISA kit upon receipt at 2-8°C. Under these conditions the kit is stable up to the expiry date stated on the box.

Note:

The specimens' storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. 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; Approved Guideline - Fourth Edition

MATERIALS**Specimen collection**

- Collect blood by venipuncture taking care to avoid haemolysis. Separate the serum from the cells within 3 hours after collection of blood. It is recommended to freeze (<-18°C) samples immediately.
- For optimal results it is recommended to draw blood as fasting morning samples (18).
- When analysing plasma, both heparia and EDTA plasma may be used.

Materials supplied

Before opening the kit, read the section on Precautions. The kit contains reagents sufficient for 96 determinations.

Streptavidin coated microtitre plate **MICROPLAT**

Microwell strips (12x8 wells) pre-coated with streptavidin. Supplied in a plastic frame.

CrossLaps Standard **CAL 0**

One vial (min. 5.0 mL/vial) of ready for use PBS buffered solution with protein stabiliser and preservative.

CrossLaps Standards **CAL 1 - 5**

Five vials (min. 0.4 mL/vial) of ready for use, CrossLaps standard in a PBS buffered solution with protein stabiliser and preservative. The exact value of each Standard is printed on the QC Report.

Control **CTRL 1 - 2**

Two vials (min. 0.4 mL/vial) of ready for use, desalted urinary antigens of human origin in a PBS buffered solution with protein stabiliser and preservative. Please refer to enclosed QC Report for control range.

Biotinylated Antibody **Ab BIOTIN**

One vial (min. 0.25 mL) of a concentrated solution of a biotinylated monoclonal murine antibody specific for degradation products of C-terminal telopeptides of Type I collagen, raised. Prepared in a buffered solution with protein stabiliser and preservative.

Peroxidase Conjugated Antibody **ENZYMCNJ**

One vial (min. 0.25 mL) of a concentrated solution of a peroxidase conjugated murine monoclonal antibody specific for degradation products of C-terminal telopeptides of Type I collagen. Prepared in a buffered solution with protein stabiliser and preservative.

Incubation Buffer **BUF**

One vial (min. 19 mL) of a ready for use buffered solution with protein stabiliser, detergent and preservative.

Substrate Solution **SUBS TMB**

One vial (min. 12 mL) of a ready for use tetramethylbenzidine (TMB) substrate in an acidic buffer. Please note that the chromogenic substrate might appear slightly blueish.

Stopping Solution **H2SO4**

One vial (min. 12 mL) of ready for use 0.18 mol/L sulfuric acid.

Washing Buffer **WASHBUF 50x**

One vial (min. 20 mL) of a concentrated washing buffer with detergent and preservative.

Sealing tape

Adhesive film for covering wells during incubation.

Materials required — not supplied

- Containers for preparing the Antibody Solution and the Washing Solution
- Precision micropipettes to deliver 50-200 µL
- Distilled water
- Precision 8 or 12 channel multipipette to deliver 100 µL, and 150 µL
- Microwell mixing apparatus
- Microtiter plate reader

ASSAY PROCEDURE

- Mix all reagents and samples before use (avoid foam)
- 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 unused immuno strips in the tightly closed foil bag with desiccant capsules.

Prior to use, prepare and equilibrate all solutions to room temperature. **Perform the assay at room temperature (18-22°C).**

1 Preparation of the Antibody Solution:

ATTENTION: Prepare the following Antibody Solution maximum 30 minutes before starting the assay. Mix the Biotinylated Antibody **Ab BIOTIN**, Peroxidase Conjugated Antibody **ENZYMCNJ** 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.**

2 One Step incubation

Pipette 50 µL of either **Standards** **CAL 0 - 5**, **Control** **CTRL 1 - 2**, or unknown samples into appropriate wells followed by 150 µL, of the **Antibody Solution**. Cover the immunostrips with sealing tape and incubate for 120±5 minutes at room temperature (18-22°C) on a microtitre plate mixing apparatus (300 rpm).

3 Washing

Wash the immunostrips 5 times manually with 300 µL diluted **Washing Buffer** (**WASHBUF 50x** diluted 1+50 in distilled water). Using an automated plate washer, follow the instructions of the manufacturer or the guidelines of the laboratory. Usually 5 washing cycles are adequate. Make sure that the wells are **completely emptied** after each manual or automatic washing cycle.

4 Incubation with chromogenic substrate solution

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 on the mixing apparatus (300 rpm). Use sealing tape. Do not pipette directly from the vial containing TMB substrate but transfer the needed volume to a clean reservoir. Remaining substrate in the reservoir should be discarded and not returned to vial TMB.

5 Stopping of colour reaction

Pipette 100 µL of the **Stopping Solution** **H2SO4** into each well.

6 Measurement of absorbance

Measure the absorbance at 450 nm with 650 nm as reference within two hours.

Limitations of the procedure

If the absorbance of a sample exceeds that of **Standard 5**, the sample should be diluted in **Standard 0** and re-analysed.

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.

RESULTS

Calculation of results

A quadratic curve fit can 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 standards 0-5 (ordinate) against the corresponding CrossLaps concentrations (abscissa). Determine the CrossLaps concentration of the controls and each patient sample by interpolation.

Example of results obtained:

Standards/ Controls/ Samples	CrossLaps conc. (ng/mL)	A ₄₅₀₋₆₅₀ (nm) Obs 1/ Obs 2	Mean A ₄₅₀₋₆₅₀ (nm)	Interpolated CrossLaps conc. (ng/mL)
Standard 0	0.000	0.066 / 0.065	0.066	
Standard 1	0.178	0.210 / 0.209	0.210	
Standard 2	0.489	0.472 / 0.448	0.460	
Standard 3	0.960	0.844 / 0.819	0.832	
Standard 4	1.902	1.598 / 1.560	1.579	
Standard 5	2.494	2.061 / 2.004	2.033	
Control 1		0.349 / 0.354	0.352	0.355
Control 2		0.918 / 0.952	0.935	1.086
Sample I		0.140 / 0.138	0.139	0.091
Sample II		0.447 / 0.439	0.443	0.469
Sample III		1.305 / 1.303	1.304	1.555

Please note: The data above are for illustration only and should not be used to calculate the results of any run.

REFERENCES

1. Bjarnason NH. & Christiansen, C. Early Response in Biochemical Markers Predicts Long-term Response in Bone Mass During Hormone Replacement Therapy in Early Postmenopausal Women. *Elsevier* 26; 561-569 (2000).
2. Bonde M, Garnero P, Fledelius C, Qvist P, Delmas PD, Christiansen C. Measurement of bone degradation products in serum using antibodies reactive with an isomerized form of an 8 aminoacid sequence of the C-telopeptide of Type I collagen. *J Bone Miner Res* 12:1028-1034 (1997).
3. Bonde M, Qvist P, Fledelius C, Riis BJ, Christiansen C. Applications of an enzyme immunoassay for a new marker of bone resorption (CrossLaps®) - follow up on hormone replacement therapy and osteoporosis risk assessment. *J Clin Endocrinol & Metab* 80:864-8 (1995).



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