

For the quantification of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in urine.

The Urine CartiLaps® ELISA is For Research Use Only. Not for use in diagnostic procedures. Nordic Bioscience Diagnostics is not responsible for any other use of the product or consequences hereof than the one specified above. Neither for misuse, e.g. use deviating from the procedure described in this manual.

Furthermore, Nordic Bioscience Diagnostics A/S is not to be made responsible for any diagnoses or conclusions made by the user or third party based on the results obtained with the Urine CartiLaps® ELISA kit nor for any consequences such interpretations may cause.

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INTRODUCTION

Intended use

The Urine CartiLaps[®] ELISA detects degradation products of C-terminal telopeptides of type II collagen (CTX-II) in human urine. The test is intended For Research Use Only. Not for use in diagnostic procedures.

Limitations

The use of the test has not been established for determination of the level of cartilage destruction.

Background

Disruption of the structural integrity of cartilage is the major histological finding in osteoarthritis and rheumatoid arthritis. Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (CTX-II) are being released into circulation and subsequently secreted into urine following degradation of cartilage. In urine, the CTX-II fragments can be quantified by Urine CartiLaps[®] ELISA.

The Urine CartiLaps[®] ELISA has been reported to be useful in prediction of progression of osteoarthritis (Reijman (2003), Garnero (2003)) and in other clinical and pre-clinical investigations (please refer to REFERENCES).

Principle of the procedure

Urine CartiLaps[®] ELISA (Christgau (2001)) is based on the competitive binding of a monoclonal antibody to urinary fragments of type II collagen or to biotinylated, synthetic peptides bound to the surface of microtitre plates coated with streptavidin.

Initially, biotinylated, synthetic peptides are bound to the surface of streptavidin-coated wells of the microtitre plate. After washing, standards, controls and urine samples are pipetted into the wells followed by addition of a solution of the monoclonal antibody. The wells are washed, and a solution of peroxidase-conjugated anti-mouse immunoglobulin (rabbit) is added to the wells. Following the second washing step, a chromogenic substrate is added to all wells and the colour reaction is stopped with sulphuric acid and the absorbance is measured.

PRECAUTIONS

The following precautions should be observed in the laboratory:

- Do not eat, drink or smoke where immunodiagnostic materials are being handled.
- Do not pipette by mouth.
- Wear gloves when handling immunodiagnostic materials.
- Do not use reagents beyond their expiration date and do not mix reagents from different lots of kits.
- Always use clean containers.

Warnings

For *in vitro* use only.

- All reagents and laboratory equipment should be handled and disposed of as if they were infectious.
- Do not use kit components beyond the expiry date and do not mix reagents from different lots.

Storage

Store the Urine CartiLaps[®] ELISA kit at 2-8°C upon receipt. Under these conditions the kit is stable up to the expiry date stated on the box.

MATERIALS

Specimen collection

It is recommended to use second morning void urine specimens, but any spot urine sample may be used. Urine samples are stable for 24 hours at 4°C and should be stored frozen (<-18°C) for longer storage. Urine samples are stable for at least 10 freeze-thaw cycles.

Prior to use, urine specimens should be shaken and sedimentation allowed for a minimum of 30 minutes.

Materials supplied

Before using the kit, please read the section on **Precautions**.

The kit contains reagents sufficient for 96 determinations.

Streptavidin coated microtitre plate (MTP)

Microwell strips (12 x 8 wells) pre-coated with streptavidin. Supplied in a plastic frame.

Urine CartiLaps[®] Standard (Vial A)

One vial (min. 3.0 mL) of a ready-for-use TRIS-buffered solution containing protein stabilizer, detergent and preservative.

Urine CartiLaps[®] Standards (Vials B-F)

Five vials (min. 0.5 mL each) of ready-for-use synthetic peptide in a TRIS-buffered solution containing protein stabilizer, detergent and preservative. The exact concentration of synthetic peptide is stated on each vial.

Urine CartiLaps[®] Control (Vial CO)

One vial (min. 0.5 mL) of ready-for-use synthetic peptide in a TRIS-buffered solution containing protein stabilizer, detergent and preservative.

Biotinylated Urine CartiLaps[®] Antigen (Vial no. 1)

One vial (min. 12.0 mL) of ready-for-use biotinylated, synthetic peptide in a PBS-buffered solution containing protein stabilizer, detergent and preservative.

Primary Antibody (Vial no. 2)

One vial (min. 12.0 mL) of ready-for-use monoclonal antibody in a TRIS-buffered solution containing protein stabilizer, detergent, preservative and a red dye.

Peroxidase Conjugated Antibody (Vial no. 3)

One vial (min. 12.0 mL) of ready-for-use peroxidase-conjugated anti-mouse immunoglobulins (rabbit) in a TRIS-buffered solution with protein stabilizer, detergent, preservative and a blue dye.

Substrate Solution (Vial TMB)

One vial (min. 12.0 mL) of a ready-for-use tetramethylbenzidine (TMB) substrate in an acidic solution. Please note that the chromogenic substrate might appear slightly bluish.

Stopping Solution (Vial ST)

One vial (min. 12.0 mL) of ready-for-use 0.18 M sulfuric acid.

Washing Solution (Vial W)

One vial (min. 20.0 mL) of a concentrated washing buffer with detergent and preservative.

Sealing tape

Adhesive film for covering wells during incubation.

Materials required - not supplied

- Container for preparing the Washing Solution.
- Precision micropipette to deliver 40 μ L.
- Precision 8 or 12-channel multipipette to deliver 100 μ L.
- Distilled water.
- Refrigerator (2-8°C).
- Microtiter plate reader for reading at both 450 nm and 650 nm.

ASSAY PROCEDURE

For optimal performance of the assay, it is important to comply with the instructions given below. Equilibrate all reagents to room temperature (18-22°C) prior to use. 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 14 wells are needed for standards and control. Place the appropriate number of strips in the plastic frame. Store unused immunostrips in the tightly closed foil bag with desiccant capsules.

Assay procedure

1 Pre-incubation

Add 100 μ L of **Biotinylated Urine CartiLaps® Antigen** (vial no. 1) to each well, cover with sealing tape, and incubate for 30 \pm 5 minutes at room temperature (18-22°C) without shaking.

2 Washing

Wash the immuno strips 5 times manually with **Washing Solution** (vial W) 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 automated washing cycle.

3 Primary incubation

Pipette 40 μ L of either **Urine CartiLaps® Standards** (vial A-F), **Control** (vial CO) or unknown urine samples into appropriate wells followed by 100 μ L **Primary Antibody** (vial no. 2). Cover the immunostrips with sealing tape and incubate for 21 \pm 3 hrs in a refrigerator (2-8°C) without shaking.

4 Washing

See step 2.

5 Secondary incubation

Add 100 μ L of the **Peroxidase-Conjugated Antibody** solution (vial no. 3) to each well. Cover the immunostrips with sealing tape and incubate for 60 \pm 5 minutes at room temperature (18-22°C) without shaking.

6 Washing

See step 2.

7 Incubation with chromogenic substrate solution

Pipette 100 μ L of the **Substrate Solution** (vial TMB) into each well, cover the immunostrips with sealing tape and incubate for 15 ± 2 minutes in the darkness at room temperature ($18-22^\circ\text{C}$) without shaking.

8 Stopping of color reaction

Pipette 100 μ L of the **Stopping Solution** (vial ST) into each well.

9 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 is lower than Standard F, it is recommended that the sample be diluted in Standard A.

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

Construct a standard curve using a four-parametric logistic curve fit, and determine the Urine CartiLaps[®] concentration of the Control (CO) and each of the patient specimens by interpolation on the curve.

Example of results obtained

Sample	Urine CartiLaps [®] concentration (ng/ml)	Abs _{450-650nm} Obs 1 / Obs 2 (Abs.)	Mean absorbance (Abs.)	Interpolated Urine CartiLaps [®] concentration (ng/ml)
Standard A	0,00	2,018/1,995	2,007	
Standard B	0,64	1,391/1,404	1,398	
Standard C	1,18	1,152/1,263	1,066	
Standard D	2,51	0,708/0,663	0,686	
Standard E	4,88	0,387/0,382	0,385	
Standard F	9,48	0,202/0,202	0,202	
Control (CO)	1,50	1,038/0,960	0,999	1,37
Sample 1		0,290/0,272	0,281	6,90
Sample 2		1,303/1,257	1,280	0,81
Sample 3		0,475/0,531	0,503	3,68

Note: The data above are for illustration only and should not be used for calculation of results.

Correction with creatinine

The CTX-II value determined as described above should be corrected with creatinine concentration.

Determine the concentration of creatinine (mmol/L) in the sample using an enzymatic colorimetric method for clinical chemistry analysers (e.g. CREA plus for Roche/Hitachi analysers) or equivalent, and perform the correction using the equation:

$$\text{Corrected CTX-II Value (ng/mmol)} = \frac{1000 \times \text{Urine CartiLaps (ng/ml)}}{\text{Creatinine (mmol/L)}}$$

Performance characteristics

Lot-to-Lot variability $\leq 7.0\%$

The lot-to-lot variability was determined by testing three urine samples in three different lots of Urine CartiLaps® ELISA.

	Mean (ng/ml)	SD (ng/ml)	CV (%)
LOW	0.47	0.033	7.0
MEDIUM	1.87	0.064	3.4
HIGH	5.44	0.136	2.5

Detection limit 0.20 ng/ml

The detection limit was determined to be 0.20 ng/ml, which is the concentration corresponding to three standard deviations below the mean of 21 determinations of the absorbance of the Urine CartiLaps® Standard A (vial A).

Precision $\leq 12.2\%$

The precision was determined using ten analytical runs, each with duplicate determinations of urine samples.

Sample	Mean (ng/ml)	Intraassay $\leq 7.8\%$		Interassay $\leq 12.2\%$	
		SD (ng/ml)	CV (%)	SD (ng/ml)	CV (%)
LOW	0.52	0.04	7.8	0.06	12.2
MEDIUM	1.84	0.08	4.6	0.20	10.8
HIGH	5.50	0.28	5.2	0.38	6.9

Dilution/Linearity 96%

The dilution recovery of the Urine CartiLaps® ELISA was determined to be 96%. 4 urine samples were appropriately diluted in Urine CartiLaps® Standard A, the concentration of CTX-II was determined in the Urine CartiLaps® ELISA and the recovery calculated by correction with the dilution factor.

	Sample 1	Sample 2	Sample 3	Sample 4	Overall RC
DF	RC%	RC%	RC%	RC%	
1x	100	100	100	100	
2x	92	104	92	98	
4x	88	105	86	93	
8x	84	104	89	108	

DF: Dilution Factor; RC: Recovery

Interference

No interference could be detected in Urine CartiLaps® ELISA with addition of the following compounds into human urine samples:

Urea	up to	30 g/L	Ibuprofen	up to	50 g/L
Creatinine	up to	10 mg/L	Acetyl-salicylic acid	up to	50 g/L
Glucose	up to	5 mg/L	Paracetamol	up to	50 g/L
Ascorbic acid	up to	5 mg/L			
Albumin	up to	50 mg/L			

Specificity

The epitope being detected in the Urine CartiLaps® ELISA is highly conserved and therefore the test can be applied to urine samples from most other species, including non-human primates, bovines, horses, pigs, rabbits, rats and mice.

Expected values

It is advisable for each laboratory to establish its own range of healthy and pathological CTX-II values. As an example, the mean values and standard deviations for various populations are given below. For further information, please refer to the reference list at the end of these instructions.

Population	Number of subjects	Age (years)	Mean CTX-II value (ng/mmol)	SD (ng/mmol)
All women	459	20-85	299	79-1137
Pre 20-30 yrs	38	20-30	464	103-2086
Pre 30-60 yrs	165	30-59	200	65-618
Post	256	46-85	363	112-1172
Pre 48-53 yrs	28	50,0±1,3	164	66-410
Post 48-53 yrs	38	51,4±1,3	318	89-1132
All male	247	22-87	278	87-895
Male 20-30 yrs	27	20-30	501	214-1171
Male 30-60 yrs	141	30-60	236	89-628
Male > 60 yrs	79	60-87	305	85-1096

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