

Urine Pre-Clinical CartiLaps® EIA

Enzymeimmunoassay for the quantitative determination of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in non-human urine and cell culture supernatants

For Research Use Only. Not for use in diagnostic procedures.

INTRODUCTION

Intended use

The Urine Pre-Clinical CartiLaps® EIA detects degradation products of C-terminal telopeptides of type II collagen (CTX-II) in non-human urine and cell culture supernatant. 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 non-human urine, the CTX-II fragments can be quantified by Urine Pre-Clinical CartiLaps® EIA, which in addition is applicable for cell culture supernatants. The corresponding test for human application, i.e. the Urine CartiLaps® EIA, has been reported to be useful in prediction of progression of osteoarthritis (Reijman (2003), Garnero (2003)) and in other clinical investigations (please refer to REFERENCES).

Principle of the procedure

The Urine Pre-Clinical CartiLaps® EIA is a modification of the Urine CartiLaps® EIA (Christgau (2001), which 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. The modification allows a broadening of the measuring range facilitating the measurement of non-human urines having a wide range of CTX-II values.

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.

Warnings

The Urine Pre-Clinical CartiLaps® EIA is for 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 the Package Insert. IDS Limited 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 practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations..

Storage

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

MATERIAL

Specimen collection

Spot urine may be used and these are stable for 24 hours at 4°C. For longer storage the urine and cell culture supernatants should be stored frozen (<-18°C). 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 **MICROPLAT**

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

Standard 0 **CAL 0**

One vial (min. 8.0 mL) of a ready-for-use TRIS-buffered solution containing protein stabilizer, detergent and preservative.

Standard 1 **CAL 1**

One vial (min. 0.4 mL) 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 the bottle label.

Control **CTRL**

One vial (min. 0.4 mL) of ready-for-use synthetic peptide in a TRIS-buffered solution containing protein stabilizer, detergent and preservative. The exact concentration is stated on the accompanying QC Report.

Biotinylated Antigen **Ag BIOTIN**

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 **Ab**

One vial (min. 18.0 mL) of ready-for-use monoclonal antibody in a TRIS-buffered solution containing protein stabilizer, detergent, preservative and a red dye.

Peroxidase Conjugated Antibody **ENZYMCONJ**

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 **SUBS 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 **H2S04**

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

Washing Solution **WASHBUF 50x**

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 10 µL.
- Precision 8 or 12-channel multipipette to deliver 100-150 µL.
- 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 18 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.

1. Preparation of standards

Standards covering the appropriate measuring range are prepared by dilution of Standard 1 **CAL** **1** in Standard 0 **CAL** **0**. Usually six two-fold dilutions of Standard 1, in addition to Standard 0 and Standard 1, will provide a suitable measuring range for most purposes.

Example:

| Standard | Preparation | Calculated CTX-II conc. (µg/L) |
|------------|-------------------------|--------------------------------|
| Standard 1 | Ready-for-use | 100.0 |
| Standard 2 | 50µL STD.1 + 50µL STD.0 | 50.0 |
| Standard 3 | 50µL STD.2 + 50µL STD.0 | 25.0 |
| Standard 4 | 50µL STD.3 + 50µL STD.0 | 12.5 |
| Standard 5 | 50µL STD.4 + 50µL STD.0 | 6.25 |
| Standard 6 | 50µL STD.5 + 50µL STD.0 | 3.13 |
| Standard 7 | 50µL STD.6 + 50µL STD.0 | 1.56 |
| Standard 0 | Ready-for-use | 0.0 |

2 Pre-dilution of test specimens (Unknown samples and control)

All specimens, unknown samples and controls **CTRL** except standards supplied with the kit must be pre-diluted 1+3 in standard 0 prior to testing (e.g. 10 µL (specimen) + 30 µL (Std. 0)).

3 Pre-incubation

Add 100 µL of Biotinylated Antigen **Ag BIOTIN** to each well, cover with sealing tape, and incubate for 30±5 minutes at room temperature (18-22°C) without shaking.

4 Washing

Wash the immuno strips 5 times manually with 300 µL Washing Solution (**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 automated washing cycle.

5 Primary incubation

Pipette 10 µL of either Standards, Control or unknown samples into appropriate wells followed by 150 µL Primary Antibody **Ab**. Cover the immunostrips with sealing tape and incubate for 21±3 hrs. in a refrigerator (2-8°C) without shaking.

6 Washing

See step 2.

7 Secondary incubation

Add 100 µL of the Peroxidase-Conjugated Antibody solution **ENZYMCNJ** to each well. Cover the immunostrips with sealing tape and incubate for 60±5 minutes at room temperature (18-22°C) without shaking.

8 Washing

See step 2.

9 Incubation with chromogenic substrate solution

Pipette 100 µL of the Substrate Solution **SUBS** **TMB** into each well, cover the immunostrips with sealing tape and incubate for 15±2 minutes in the darkness at room temp. (18-22°C) without shaking.

10 Stopping of color reaction

Pipette 100 µL of the Stopping Solution **H2S04** into each well.

11 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 1, it is recommended that the sample be diluted in Standard 0.

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 CartiLaps concentration of the Control and each of the samples by interpolation on the curve.

Example:

| Sample | CTX-II concentration (µg/L) | Absorbance Abs _{450-650 nm} Obs 1 / Obs 2 (Abs.) | Mean (Abs.) | Interpolated CTX-II concentration (µg/L) | Conc. Corrected for 4x dilution (µg/L) |
|------------|-----------------------------|--|-------------|--|--|
| Standard 0 | 0.00 | 1.962/1.904 | 1.933 | | |
| Standard 7 | 1.4 | 1.541/1.681 | 1.611 | | |
| Standard 6 | 2.9 | 1.329/1.322 | 1.326 | | |
| Standard 5 | 5.8 | 0.987/0.948 | 0.968 | | |
| Standard 4 | 11.5 | 0.603/0.582 | 0.593 | | |
| Standard 3 | 23.0 | 0.357/0.352 | 0.355 | | |
| Standard 2 | 46.0 | 0.178/0.178 | 0.178 | | |
| Standard 1 | 92.0 | 0.098/0.094 | 0.096 | | |
| Control | | 0.312/0.292 | 0.302 | 26.3 | |
| Sample I | | 0.708/0.716 | 0.708 | 9.3 | 37.2 |
| Sample II | | 0.263/0.297 | 0.280 | 28.7 | 114.8 |
| Sample III | | 0.140/0.134 | 0.137 | 62.6 | 250.4 |

Please 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:

$$\text{Corrected CTX-II Value (µg/mmol)} = \frac{\text{CartiLaps (µg/L)}}{\text{Creatinine (mmol/L)}}$$

Performance characteristics

Detection limit 0.75 µg/L

The detection limit was determined to 0.75 µg/L, which is the concentration corresponding to three standard deviations below the mean of 21 determinations of the absorbance of the Standard 0.

Precision <8.1%

| Intraassay < 4.6% (n=10) | | | Interassay < 8.1% (n=10) | | |
|--------------------------|-----|-----|--------------------------|-----|-----|
| Mean | SD | CV | Mean | SD | CV |
| 10,2 | 041 | 4.4 | 10.2 | 0.8 | 8.1 |
| 30.4 | 1.2 | 4.1 | 30.4 | 2.0 | 6.6 |
| 62.1 | 5.8 | 4.6 | 62.1 | 4.5 | 7.2 |

Dilution/Linearity 97%

The dilution recovery of the Urine Pre-Clinical CartiLaps® EIA was determined to 97%. Urine samples with CTX-II values inside the measuring range were appropriately diluted in Standard 0, the concentration of CTX-II was determined in the Urine Pre-Clinical CartiLaps® EIA and the recovery calculated by correction with the dilution factor.

| DF | Sample 1 RC% | Sample 2 RC% | Sample 3 RC% | Sample 4 RC% | Overall RC |
|----------|--------------|--------------|--------------|--------------|------------|
| 4x ~neat | 100 | 100 | 100 | 100 | 97% |
| 2x | 100 | 92 | 91 | 86 | |
| 8x | 124 | 81 | 77 | 104 | |
| 16x | 97 | 105 | 83 | 111 | |

DF: Dilution Factor; RC: Recovery

Specificity

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

CLINICAL DATA









Expected values

It is advisable for each laboratory to establish its own reference ranges. As an example, the mean values and standard deviations for various species are given below.

| Species | Number of samples | Mean CTX-II value (µg/mmol) | Range (µg/mmol) |
|--------------------------|-------------------|-----------------------------|-----------------|
| Dog (study 1) | 169 | 69.5 | 4.1-260.4 |
| Dog (study 2) | 75 | 666 | 26.6-2132 |
| Guinea Pig (study 3) | 172 | 43.9 | 1.1-231.2 |
| Guinea Pig (study 4) | 60 | 388 | 57.4-840 |
| Rabbit (study 5) | 65 | 41.9 | 7.6-114 |
| Rabbit (study 6) | 165 | 625.2 | 60.8-1745 |
| Rat (study 7) | 56 | 24.3 | 7.8-68.2 |
| Rat (study 8) | 45 | 95.4 | 35.5-135 |
| Cell Culture Supernatant | 54 | 69.8 | 2.0-237.0 |

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|---|---|---|---|
|  EXP | GB <i>Use By</i> DE <i>Verwendbar bis</i> ES <i>Fecha de caducidad</i> IT <i>Utilizzare entro</i> FR <i>Utiliser jusque</i> NL <i>Houdbaar tot</i> DK <i>Holdbar til</i> CZ <i>Použitelné do</i> SK <i>Použitelné do</i> GR <i>Ημερομηνία λήξης</i> PT <i>Prazo de validade</i> HU <i>Felhasználható</i> SE <i>Använd före</i> PL <i>Użyć przed</i> |  LOT | GB <i>Batch code</i> DE <i>Chargenbezeichnung</i> ES <i>Código de lote</i> IT <i>Codice del lotto</i> FR <i>Code du lot</i> NL <i>Lot nummer</i> DK <i>Lotnummer</i> CZ <i>Číslo šarže</i> SK <i>Číslo šarže</i> GR <i>Αριθμός Παρτίδας</i> PT <i>Código do lote</i> HU <i>Sarzsorszám</i> SE <i>Lot nummer</i> PL <i>Kod partii</i> |
|  REF | GB <i>Catalogue number</i> DE <i>Bestellnummer</i> ES <i>Número de catálogo</i> IT <i>Numero di catalogo</i> FR <i>Référence du catalogue</i> NL <i>Catalogus nummer</i> DK <i>Katalognummer</i> CZ <i>Katalogové číslo</i> SK <i>Katologové číslo</i> GR <i>Αριθμός καταλόγου</i> PT <i>Referência de catálogo</i> HU <i>Katalógusszám</i> SE <i>Katalognummer</i> PL <i>Numer katalogowy</i> |  | GB <i>Manufacturer</i> DE <i>Hersteller</i> ES <i>Fabricante</i> IT <i>Fabbricante</i> FR <i>Fabricant</i> NL <i>Fabrikant</i> DK <i>Producent</i> CZ <i>Výrobce</i> SK <i>Výrobca</i> GR <i>Κατασκευαστής</i> PT <i>Fabricante</i> HU <i>Gyártó</i> SE <i>Tillverkare</i> PL <i>Producent</i> |
|  | GB <i>Contains sufficient for <n> tests</i> DE <i>Inhalt ausreichend für <n> Prüfungen</i> ES <i>Contenido suficiente para <n> ensayos</i> IT <i>Contenuto sufficiente per "n" saggi</i> FR <i>Contenu suffisant pour "n" tests</i> NL <i>Inhoud voldoende voor "n" testen</i> DK <i>Indeholder tilstrækkeligt til "n" test</i> CZ <i>Lze použít pro <n> testů</i> SK <i>Obsah postačuje na <n> stanovení</i> GR <i>Περιεχόμενο επαρκές για «ν» εξετάσεις</i> PT <i>Conteúdo suficiente para "n" ensaios</i> HU <i>A doboz tartalma <n> vizsgálat elvégzéséhez elegendő</i> SE <i>Räcker till "n" antal tester</i> PL <i>Wystarczy na wykonanie <n> testów</i> |  IVD | GB <i>In Vitro Diagnostic Medical Device</i> DE <i>In-Vitro-Diagnostikum</i> ES <i>Producto sanitario para diagnóstico in vitro</i> IT <i>Dispositivo medico-diagnostico in vitro</i> FR <i>Dispositif médical de diagnostic in vitro</i> NL <i>Medisch hulpmiddel voor in-vitro diagnostiek</i> DK <i>Medicinsk udstyr til in vitro-diagnostik</i> CZ <i>In Vitro diagnostický zdravotnický prostředek</i> SK <i>Zdravotnícka pomocka in vitro</i> GR <i>Ιn Vitro Διαγνωστικό Ιατροτεχνολογικό προϊόν</i> PT <i>Dispositivo médico para diagnóstico in vitro</i> HU <i>In vitro diagnosztikum</i> SE <i>Medicintekniska produkter för in vitro diagnostik</i> PL <i>Wyrób do diagnostyki In Vitro</i> |
|  | GB <i>Temperature limitation</i> DE <i>Temperaturbegrenzung</i> ES <i>Límite de temperatura</i> IT <i>Limiti di temperatura</i> FR <i>Limites de température</i> NL <i>Temperatuurlimiet</i> DK <i>Temperaturbegrænsning</i> CZ <i>Teplotní rozmezí od do</i> SK <i>Teplotné rozmedzie od do</i> GR <i>Περιορισμοί θερμοκρασίας</i> PT <i>Limites de temperatura</i> HU <i>Hőmérsékletartomány</i> SE <i>Temperaturbegränsning</i> PL <i>Przestrzegać zakresu temperatury</i> |  | GB <i>Consult Instructions for Use</i> DE <i>Gebrauchsanweisung beachten</i> ES <i>Consulte las instrucciones de uso</i> IT <i>Consultare le istruzioni per l'uso</i> FR <i>Consulter les instructions d'utilisation</i> NL <i>Raadpleeg de gebruiksaanwijzing</i> DK <i>Se brugsanvisning</i> CZ <i>Viz návod k použití</i> SK <i>Vid' návod na použitie</i> GR <i>Συμβουλευτείτε τις οδηγίες χρήσης</i> PT <i>Consulte as instruções de utilização</i> HU <i>Nézze meg a Használati utasítást</i> SE <i>Se handhavandebeskrivningen</i> PL <i>Sprawdź w instrukcji obsługi</i> |



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