immunodiagnosticsystems

Serum Pre-Clinical CartiLaps® (CTX-II) ELISA

Enzymeimmunoassay for the quantitative determination of degradation products of C-terminal telopeptides of type II collagen (CTX-II) in animal serum

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

REF AC-08F1 Σ 96

INTRODUCTION

Intended use

The Serum Pre-Clinical CartiLaps® (CTX-II) ELISA detects degradation products of C-terminal telopeptides of type II collagen (CTX-II) in animal serum. 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 serum, the CTX-II fragments can be quantified by Serum Pre- Clinical CartiLaps® (CTX-II) ELISA. The Serum Pre-Clinical CartiLaps® (CTX-II) ELISA has been reported to be useful for rat, mice and rabbit specimen.

Principle of the procedure

Serum Pre-Clinical CartiLaps® (CTX-II) ELISA is based on binding of two identical monoclonal antibodies to cross-linked serum fragments of type II collagen. Initially, biotinylated monoclonal antibody are bound to the surface of streptavidin-coated wells of the microtitre plate. After washing, standards, controls, serum samples and Incubation buffer are pipetted into the wells. After incubation the wells are washed, and a solution of peroxidase-conjugated monoclonal antibody is added to the wells. Following the third 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

Storage

Store the Serum Pre-Clinical CartiLaps® (CTX-II) ELISA kit at 2-8°C upon receipt. 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

Warnings

The Serum Pre-Clinical CartiLaps® (CTX-II) ELISA 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. Immunodiagnostic Systems 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.

Sodium Azide

Some reagents in this kit contain sodium azide as a preservative, 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.

Calibrators CAL and Controls CTRL contain sodium azide (NaN3) >1% (w/w).

Classification according to Regulation (EC) CLP:

Acute toxicity (oral), Category 4
Hazardous to the aquatic environment – Chronic Hazard, Category 3

Warning

Contains Sodium Azide

Hazard statements:

H302 - Harmful if swallowed.
H412 - Harmful to aquatic life with long lasting effects.

Precautionary statements

P264 - Wash hands, forearms and face thoroughly after handling.

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.. Do not use reagents beyond their expiration date and do not mix reagents from different lots of kits.

MATERIAL

Specimen collection

The Serum Pre-Clinical CartiLaps® (CTX-II) ELISA is intended for use with serum samples. However in-house data has verified that CTX-II can be detected in synovial fluid and EDTA plasma as well. Collect blood 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. Serum samples are stable for 3 freeze-thaw cycles. Prior to use the serum samples should be shaken.

Materials supplied

Before opening the kit, read the section on Precautions. The kit contains reagents sufficient for 96 determinations. For reconstitution of lyophilized material add appropriate volume of distilled water and leave for 10 minutes before mixing. Make sure to avoid foam.

Streptavidin coated microtitre plate MICROPLAT

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

CartiLaps Standard 0 CAL 0

One vial (min. 9.0 mL/vial) of ready for use PBS buffered solution with protein stabiliser and preservative.

CartiLaps Standard 1 CAL 1

One vial (lyophilized) containing Foetal Calf Serum (FCS) in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with min. 0.5 mL of distilled or deionised water. The exact concentration is stated on each vial. The standards must be stored below -18°C after use, and should only be frozen and thawed twice.

Control CTRL

One vial (lyophilized) containing FCS in a PBS-buffered solution with protein stabilizer and preservative. Reconstitute with min. 0.5 mL of distilled or deionised water. The exact concentration is stated on the QC Report provided with the kit. The control must be stored below -18°C after use, and should only be frozen and thawed twice.

Biotinylated Antibody AB BIOTIN

One vial (min. 0.20 mL) of a concentrated solution of a biotinylated monoclonal murine antibody specific for degradation products of C-terminal telopeptides of type II collagen (CTX-II). Prepared in a buffered solution with protein stabiliser and preservative.

Peroxidase Conjugated Antibody ENZYMCONJ

One vial (min. 0.20 mL) of a concentrated solution of a peroxidase conjugated murine monoclonal antibody specific for degradation products of C-terminal telopeptides of type II collagen (CTX-II). Prepared in a buffered solution with protein stabiliser and preservative.

Incubation Buffer BUF

One vial (min. 25 mL) of a ready for use buffered solution with protein stabiliser, detergent and preservative.

Biotinylated Antibody Buffer AB BUF

One vial (min. 12.5 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 sligthy 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. Dilute 1+50 in distilled water before use.

Sealing tape

Adhesive film for covering wells during incubation.

Materials required – not supplied

- Containers for preparing; Standards, Antibody Solutions and Washing Solution
- Precision micropipettes to deliver 25-200 µL
- Distilled or deionized water
- Precision 8 or 12 channel multipipette to deliver 100 µL
- Microwell mixing apparatus
- ELISA plate reader with 450 nm, and 650 nm as reference wavelength

ASSAY PROCEDURE

Mix all reagents and samples before use (avoid foam). Prior to use, prepare and equilibrate all solutions to room temperature. Perform the assay at room temperature (18 22°C).

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 immuno strips 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, four 2.5 times dilutions of Standard 1, in addition to Standard 0 and Standard 1, will provide a suitable measuring range for most purposes.

Standard	Preparation	Calculated CTX-II conc. (pg/mL)
Standard 1	Ready-for-use	247.6
Standard 2	50µL STD.1 + 75µL STD.0	99.0
Standard 3	50µL STD.2 + 75µL STD.0	39.6
Standard 4	50µL STD.3 + 75µL STD.0	15.8
Standard 5	50µL STD.4 + 75µL STD.0	6.3
Standard 0	Ready-for-use	0.0

2 Pre-incubation

Prepare the following Biotinylated Antibody Solution before starting the assay. Mix the Biotinylated Antibody **AB BIOTIN** and Biotinylated Antibody Buffer **AB BUF** in the volumetric ratio 1+100 in an empty container. Mix carefully and avoid formation of foam. Prepare a fresh solution before each run of the assay.

Add 100 µL of Biotinylated Antibody Solution to each well, cover with sealing tape, and incubate for 30±5 minutes at room temperature (18-22°C) on a microtitre plate mixing apparatus (300 rpm).

3 Washing

Wash the immuno strips 5 times manually with 300 µL Washing Solution **WASHBUF 50x** diluted 1+50 in distilled or deionized 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.

4 Incubation of samples and standards

Pipette 25 µL of Standards (vial 0-5), Control **CTRL** or unknown samples into appropriate wells followed by 100 µL Incubation Buffer **BUF** Cover the immunostrips with sealing tape and incubate for 60±5 minutes at room temperature (18-22ºC) on a microtitre plate mixing apparatus (300 rpm).

5 Washing

See step 3.

6 Secondary incubation

Prepare the following Peroxidase Conjugated Antibody Solution before use. Mix the Peroxidase Conjugated Antibody **ENZYMCONJ** and Incubation Buffer **BUF** in the volumetric ratio 1+100 in an empty container. Mix carefully and avoid formation of foam. Prepare a fresh solution before each run of the assay. Add 100 µL of Peroxidase-Conjugated Antibody to each well, cover with sealing tape, and incubate for 60±5 minutes at room temperature (18-22ºC) on a microtitre plate mixing apparatus (300 rpm).

7 Washing

See step 3.

8 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 temperature (18-22°C) on a microtitre plate mixing apparatus (300 rpm).

9 Stopping of colour reaction

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

10 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 higher than Standard 1 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 4-parametric 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 CartiLaps concentrations (abscissa). Determine the CartiLaps concentration of the controls and each patient sample by interpolation.

Example:

Standards/ Controls/ Specimen	CartiLaps conc. (pg/mL)	$A_{450-650}$ Obs 1/ Obs 2	Mean $A_{450-650}$ (nm)	Interpolated CartiLaps conc. (pg/mL)
Standard 0	0.0	0.121 / 0.112	0.117	
Standard 5	6.9	0.174 / 0.168	0.171	
Standard 4	17.3	0.253 / 0.252	0.253	
Standard 3	43.3	0.428 / 0.446	0.437	
Standard 2	108.2	0.946 / 0.924	0.935	
Standard 1	270.5	2.011 / 1.964	1.988	
Control		0.684 / 0.687	0.686	74.8
Sample I		0.230 / 0.229	0.230	15.2
Sample II		0.473 / 0.467	0.470	46.5
Sample III		0.880/ 0.848	0.864	98.8

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

Performance characteristics

Detection limit: 3.7 pg/mL

This is the concentration corresponding to three standard deviations above the mean of 21 determinations of the blank (Serum Pre-Clinical CartiLaps Standard 0).

Precision <5.8%

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

Sample	Mean level (pg/mL)	Intraassay <7.8%		Interassay <12.2%	
		SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
Low	11.67	0.96	5.8	0.62	3.7
Medium	49.09	1.83	3.7	0.47	1.0
High	107.44	5.40	5.0	2.61	2.4

Dilution/Linearity

The Serum Pre-Clinical CartiLaps® (CTX-II) ELISA is linear in the range 3.7 pg/mL to 300.0 pg/mL.

Serum samples with the concentration of 38.8 - 57.0 pg/mL CartiLaps were diluted with standard 0 and the concentration of CartiLaps were determined with Serum Pre-Clinical CartiLaps® (CTX-II) ELISA. The serum neat sample is set to 100%.

The data below is calculated from 1 run:

Dilutions	Sample 1	Sample 2	Sample 3	Sample 4	Overall RC (%)
Serum (%)	Standard 0 (%)	RC%	RC%	RC%	
100	0	100	100	100	
80	20	106	99	105	101
60	40	103	106	101	
40	60	90	115	104	
20	80	101	110	98	
10	90	82	97	no data	

RC: Recovery

CLINICAL DATA

Expected values

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

Species	Mean CTX-II value (pg/mL)
Rats, 8 weeks normal	26.7
Mice, 8 weeks normal	24.0
Mice, 10 weeks normal	11.3

REFERENCES

1. Christgau S. et al., Suppression of Elevated Cartilage Turnover in Postmenopausal Women and in Ovariectomized Rats by Estrogen and a Selective Estrogen Receptor Modulator (SERM). *Menopause (2004); 11:508-518.*
2. Christgau S. et al., Collagen type II degradation products in urine as an index of cartilage degradation. *Bone (2001); 29: 209-215.*
3. Høegh-Andersen P. et al., Ovariectomized Rats as a Model of Postmenopausal Osteoarthritis. Validation and Application. *Annals of Rheum Dis. (2004); 6(2): R169-80.*
4. Reijman M. et al., A new marker for osteoarthritis: cross-sectional and longitudinal approach. *Arthritis & Rheum. (2004); 50:2471-2478.*
5. Roy-Beaudry M. et al., Entothelin-1 promotes osteoarthritic cartilage degradation via mmp-1 and mmp- 13 induction. *Arthritis & Rheum (2003); 48:2855-2864.*

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

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