Urinary Collagen IV EIA

Enzyme Immunoassay

Instructions for Use

FOR RESEARCH USE ONLY

Not for use in Diagnostic Procedures

ACTIF

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INTENDED USE

The Urinary Collagen IV EIA provides a method for the quantitative determination of collagen IV in human urine. Urinary Collagen IV EIA is for research use only. Not for use in diagnostic procedures.

BACKGROUND

Type IV collagen (IV•C), is a major component of the basement membrane (BM), and it is considered to constitute its basic framework. Urinary collagen IV levels are elevated in a variety of renal pathologies[1], particularly diabetic nephropathy[2-8]. Urinary collagen IV is significantly higher in patients with non-insulin dependent diabetic mellitus (NIDDM) than in normal subjects and urinary collagen IV levels correlate with the deposition of collagen IV in the kidney[2]. In diabetic subjects, urinary collagen IV is significantly increased in patients with microalbuminuria or overt proteinuria as compared to those with normoalbuminuria[3] (Figure 1). Moreover, in diabetics with normoalbuminuria, those with elevated urinary collagen IV were at an increased risk for progression to microalbuminuria[4]. Intensive therapy of diabetic nephropathy can slow the temporal increase in urinary collagen IV in diabetics indicating the potential of urinary collagen IV for studying the renal effects of new therapies[5]. These results suggest that the measurement of UIV•C might provide a useful biomarker for studying diabetic nephropathy.

In the field of renal transplantation, renal collagen IV levels are increased[9] and increased urinary collagen IV levels are found in acute renal rejection[10], indicating the potential value of urinary collagen IV in studying these conditions.

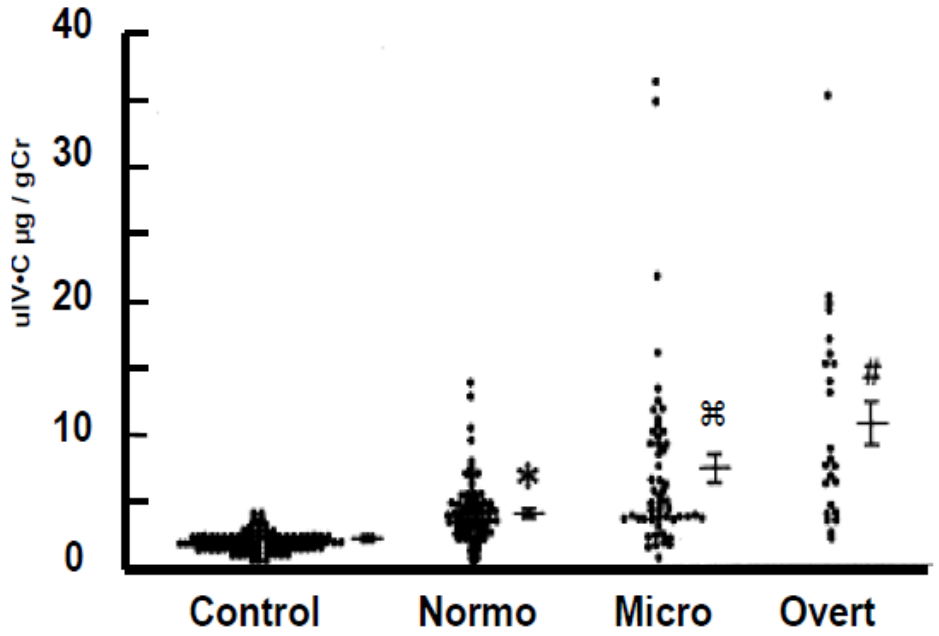

Figure. 1. Urinary collagen IV in diabetic subjects with various stages of diabetic nephropathy3. Control; n=89, Normo (normoalbuminuria); n=62, Micro (microalbuminuria); n=53, Overt (overt proteinuria); n=24, mean±SE. □ $p<0.01$ vs Control, $p<0.05$ vs Normo, # $p<0.05$ vs Micro.

ASSAY PRINCIPLE

Collagen IV in the sample is bound simultaneously by a solid-phase monoclonal antibody and a monoclonal antibody enzyme conjugate, each directed at different antigenic sites. This results in the collagen IV molecule being sandwiched between the solid phase and enzymelabelled antibodies. After removing unbound enzyme-labelled antibody and sample,the plate is incubated with enzyme substrate.
The resultant colour development is directly proportional to the amount of collagen IV in the sample.

COMPONENTS

1. Antibody coated Microassay plate: 12x8 well strips coated with IgG directed against human collagen IV. READY TO USE

2. Collagen IV Calibrators: Purified collagen IV in phosphate buffer (pH 6.0) with Bovine Serum Albumin (BSA). 0.8, 3.2, 12.5 and 50 µg/L. (1 mL each). Contains 30 mg/L Proclin 300 as preservative. READY TO USE

3. Assay Buffer: Phosphate buffer (pH 7.0) containing bovine serum albumin and horse serum (10 mL). Contains 30 mg/L Proclin 300 preservative.
READY TO USE

4. Conjugate: Anti-collagen IV mouse Fab' conjugated to horseradish peroxidase (20 mL). Contains 30 mg/L Proclin 300 as preservative.
READY TO USE

5. Wash Concentrate: 10x Conc. Phosphate buffer with Tween 20 (PBT), (2 bottles of 50 mL). Contain 30 mg/L Proclin 300 as preservative. CONCENTRATE

6. Substrate: Stabilised liquid TMB solution (15 mL).READY TO USE

7. Stop Solution: 1M Sulphuric Acid (15 mL).READY TO USE

8. Plate Seal: 1 sheet

9. Instructions for use

10. Uncoated microassay plate

PRECAUTIONS

SAFETY

- The Urinary Collagen IV EIA kit is intended for use by qualified laboratory staff only.
- The kit contains material of human origin that has been tested and found to be negative for Hepatitis B surface antigen, Hepatitis C and HIV antibodies. However, since no test can provide complete assurance, treat all materials as potentially infectious.
- The Stop Solution contains sulphuric acid which is corrosive. Avoid contact with the skin and eyes. If contact occurs, rinse off immediately with water and seek medical advice.
- The Substrate contains TMB that may irritate the skin and mucous membranes. Any substrate which comes in contact with the skin should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Residues of chemicals and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by the mouth and never eat or drink at the laboratory workbench.

PROCEDURAL

- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges specified may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Reagent delivery should be aimed at midpoint of the side of the wells, taking

care not to scratch the side with the pipette tip.

- Do not allow the wells to dry at any stage during the assay procedure.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution.
- Ensure Wash Concentrate is mixed thoroughly and no crystals remain before reconstitution.
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background colour in the assay.
- Allow all reagents to come to room temperature (20-27°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay, an identification and distribution plan should be established.

STABILITY AND STORAGE

1. All kit reagents should be stored at 2-8°C and are stable as supplied until the expiry date shown.
2. Prepared Wash Solution (PBT) is stable for up to one month at 2-8°C
3. Plate assay wells should be stored in sealed bags with desiccants at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.
4. EKF Diagnostics Urinary Collagen IV Sample Collecting Tubes are stable at room temperature until the expiry date shown. Return unused tubes to the storag

ADDITIONAL MATERIALS REQUIRED

1. 20 µL and 150 µL micropipettes and a 100-150 µL multichannel pipette
2. Microassay strip washing system
3. ELISA plate reader capable of measuring at 450nm with reference at 630nm if available
4. 1 L beaker

5. Timer
6. Liquid trough
7. Deionised/Distilled water
8. Graduated cylinder (500 mL)

PREPARATION OF REAGENTS

WASH SOLUTION (PBT)

Perform a 1/10 dilution of Wash Concentrate adding, for example, 10mL Wash Concentrate to 90 mL deionised water as required. Prepare only the volume of Wash Solution required for the assay. Each row of assay wells requires 15 mL of Washing Solution.

Ensure salt crystals are dissolved prior to dilution.

Gentle warming of Wash Concentrate at 37°C for 30 minutes will aid dissolution of salt crystals.

SAMPLE COLLECTION AND STORAGE

Collagen IV precipitates out of urine upon standing leading to falsely low results. This can be prevented by the addition of a stabilising buffer to the urine after which the urine can be stored[11]. The EKF Diagnostics Urinary Collagen IV Sample Collecting Tubes provide a simple and reproducible means of collecting urine samples for the assay of collagen IV.

Transfer urine to the collection tube using a Pasteur pipette. Fill the tube to the line indicated, then mix thoroughly. As collagen IV is absorbed by urinary precipitates that form during storage, collect fresh urine in the EKF Diagnostics Urinary Collagen IV EIA Urine Collection Tubes. Urine samples must be transferred to the collection tubes on the day of collection. Samples must be transferred to the collection tubes, even if they are not to be stored. To facilitate compensation for dieresis, it is recommended that a simultaneous sample be taken for urinary creatinine.

After addition to the urine tubes, samples can be stored at 2-8^{o}C for one week or nine months at –20^{o}C. If samples have been frozen, it is essential to mix thoroughly to dissolve any precipitates. Repeated freeze thawing of samples should be avoided.

Calibrators should be prepared immediately before use. Do not store. The diluted calibrators are stable for at least 6 hours at 2-8° C.

SAMPLE HANDLING AND STORAGE

Samples can be stored at 2-8^{o}C for one week. Samples may be stored at –20^{o}C for 12 months. Repeated freeze-thawing of samples should be avoided.

ASSAY PROCEDURE

NOTE: All reagents should be allowed to reach room temperature prior to commencement of assay.

1. IMMUNOREACTION

1.1 Prepare Wash Solution as described in "Preparation of Reagents".

1.2 Place required number of Microassay wells in the assay plate (10 for the

Calibrators plus two for each sample).

1.3 Add **150 µL** Conjugate to each well using a multichannel pipette.

1.4 Add Calibrators **(0 (Assay buffer), 0.8, 3.2, 12.5 and 50 µg/L)** and samples **(50 µL/well)**, in duplicate, to the Microassay plate.

1.5 Cover the Microassay plate with the plate seal and incubate at room temperature (20-27°C) for 24 hours.

1.6 Remove plate seal cover and wash each strip five times **(350 µL/well)** with Wash Solution. When complete, firmly tap the plate against a paper towel to ensure complete removal of wash fluid from wells.

2. COLOUR DEVELOPMENT

2.1 Add **100µL** Substrate/well using a multichannel pipette and incubate at room temperature (20-27$_o$C) for exactly one hour.

3. STOP

3.1 Add **100 µL** Stop Solution/well using a multichannel pipette. Ensure complete mixing of Substrate and Stop Solution.

3.2 Read **immediately** at 450nm using 630nm as reference (if available).

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each calibrator and sample.

2. Plot a Calibration curve of $A_{450/630nm}$ versus collagen IV (µg/L) on a log-log scale.

3. Read the collagen IV (µg/L) indicated by the mean absorbances of the samples from the calibration curve.

4. If the samples have been diluted, multiply the calculated [collagen IV] by the appropriate dilution factor in order to obtain the actual [collagen IV].

PERFORMANCE CHARACTERISTICS

NORMAL RANGE

Based on healthy Japanese volunteers, 95% confidence limits for urinary collagen IV are:

		Collagen IV µg/g creatinine	N
Early morning urine	30-39 years	<4.0	122
	>40 years	<4.9	64
Spot urine	<21 years	<7.3	390

LIMIT OF DETECTION

The detection limit of EKF Diagnostics Urinary Collagen IV EIA is 0.8 µg/L.

MEASURING RANGE

The calibration curve range covers the range 0.8-50 µg/L. This range may be extended by increasing sample dilution.

SPECIFICITY

The Urinary Collagen EIA IV is highly specific for the detection of collagen IV. Cross reactivity is less than 2% with Collagen II and less than 0.5% with other forms of collagen When reading from the standard curve the A_{450nm} value of the 1000 µg/L standard.should be >0.6 OD.

SENSITIVITY

When reading from the standard curve the A_{450nm} value of the 0.8 □g/L standard should be 0.01 – 0.06, and the 50 □g/L standard should be >0.8.

INTERFERENCE

No significant interference has been observed in this assay with creatinine, haemolytic or icteric samples.

- Creatinine: Less than 10% interference up to 3 g/L in sample.
- Haemolysis: Less than 10% interference up to 4.8 g/L haemoglobin.
- Icteris: Less than 10% interference up to 0.2 g/L bilirubin.

DILUTION - RECOVERY

Dilution of samples containing high levels of collagen IV gave the following results:

Sample	Dilution								
	1 / 2			1 / 4			1 / 8		
	Expected µg/L	Obtained µg/L	Recovery %	Expected µg/L	Obtained µg/L	Recovery %	Expected µg/L	Obtained µg/L	Recovery %
A	11.6	11.0	95	5.8	5.2	90	2.9	2.6	90
B	13.4	12.7	95	6.7	6.4	96	3.3	3.1	94
C	5.1	5.4	106	2.6	2.5	96	1.3	1.3	100

REPRODUCIBILITY

Intra-assay variation of Urinary Collagen IV EIA

Sample	$\bar{X}$ [Collagen IV] µg/L	SD	% CV	N
Low	2.5	0.07	2.8	8
Medium	6.2	0.13	2.1	8
High	10.8	0.29	2.7	8

Inter-assay variation of Urinary Collagen IV EIA

Sample	$\bar{X}$ [Collagen IV] µg/L	SD	%CV	N
Low	2.4	0.05	2.1	4
Medium	6.0	0.15	2.0	4
High	20.8	1.52	7.3	4

Inter-batch Variation of Urinary Collagen IV EIA calculated for four batches of kits

Sample	$\bar{X}$ [Collagen IV] µg/L	SD	%CV	N
Low	3.2	0.18	5.6	4
Medium	5.8	0.11	1.9	4
High	17.0	0.52	3.1	4

EXAMPLE OF CALIBRATION CURVE

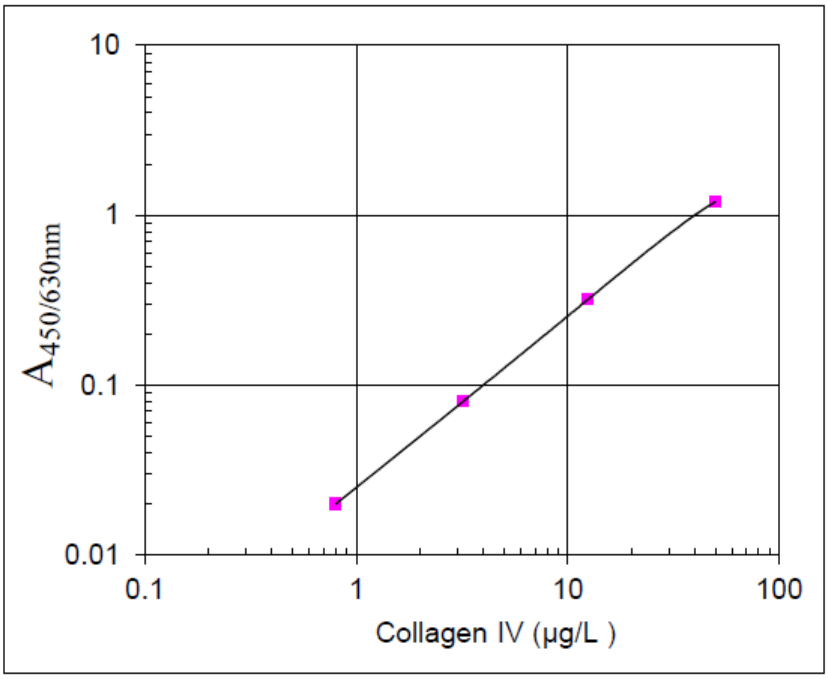

Figure 2: Typical Calibration curve obtained using the EKF Diagnostics Urinary Collagen IV EIA. Plot of $A450_{/630nm}$ versus [Collagen IV] µg/L. Assay range is 0.8 – 50 µg/L

REFERENCES

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4) *Miwa Morita et al. Association of Urinary Type IV Collagen With GFR Decline in Young Patients With Type 1 Diabetes.* Am J Kidney Dis. 2011 Dec;58(6):915-920.

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