KAMIYA BIOMEDICAL COMPANY

Human Collagen IV ELISA, Urine

For the quantitative determination of human Type IV Collagen in urine

Cat. No. KT-036

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT
The **K-*ASSAY*®** Human Collagen IV ELISA, Urine is an enzyme immunoassay for the quantitative determination of human type IV collagen in urine. For research use only, not for use in diagnostic procedures.

INTRODUCTION
The **K-*ASSAY*®** Human Collagen IV ELISA, Urine is a one-step sandwich ELISA for type IV collagen in urine that uses a pair of monoclonal antibodies to recognize different sites on the molecule of collagen IV. Type IV collagen is a major component of the basement membrane and is considered to constitute its basic framework.

PRINCIPLE
The **K-*ASSAY*®** Human Collagen IV ELISA, Urine is designed for the assay of urinary collagen IV. It is a solid phase one-step sandwich ELISA. Collagen IV in the sample is bound simultaneously by a solid phase monoclonal antibody (mAb) and a horseradish peroxidase (HRP)-mAb conjugate directed at different antigenic sites. This results in the collagen IV molecule being sandwiched between the solid phase and HRP-labelled antibodies. After removing unbound HRP-mAb conjugate and sample, the plate is then incubated with chromogenic substrate 3,3',5,5'-tetramethylbendizine (TMB), resulting in the development of a color. The color developed is directly proportional to the amount of collagen IV present in the sample.

COMPONENTS
1. Antibody coated Microwell Plate: 12 X 8-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 protein stabilizer. Calibrators at 0.8, 3.2, 12.5 and 50 µg/L of collagen IV (1 mL each). Contains 30 mg/L of Proclin 300 as a preservative. READY TO USE.
3. Assay Buffer: Phosphate Buffer (pH 7.0) with protein stabilizer (10 mL). Contains 30 mg/L of Proclin 300 as a preservative. READY TO USE.
4. Conjugate: Anti-collagen IV mouse Fab' conjugated to horseradish peroxidase (20 mL). Contains 30 mg/L of Proclin 300 as a preservative. READY TO USE.
5. Wash Solution Concentrate: 10X concentrated phosphate buffer with Tween 20 (PBT) (2 x 50 mL). Contains 30 mg/L of Proclin 300 as a preservative. CONCENTRATE.
6. Chromogenic Substrate: Stabilized liquid TMB solution (15 mL). READY TO USE.
7. Stop Solution: 1 M sulfuric acid (15 mL). READY TO USE.
8. Plate Seal
9. Collection Tubes: IN A SEPARATE PACKAGE. Urinary Collagen IV ELISA collection tubes containing urinary collagen IV stabilizing buffer (45 tubes). READY TO USE.

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED
- Microplate reader capable of measuring at 450 nm with reference at 630 nm if available.
- 50 µL precision pipette and a multi-channel pipette 100-150 µL with disposable tips.
- Microplate washing system.
- 1 L beaker
- Timer
- Liquid trough
- De-ionized/Distilled water
- Graduated cylinder (500 mL)
- Pasteur pipette

PROTOCOLS

Preparation of Reagents

Wash Solution (PBT)

Perform a 1:10 dilution of Wash Solution Concentrate by adding, for example, 10 mL Wash Solution Concentrate to 90 mL de-ionized water as required. Prepare only the volume of Wash Solution required for the assay. Each row of assay wells requires 15 mL of Wash Solution. **Ensure salt crystals are dissolved prior to dilution.** Gentle warming of Wash Solution Concentrate at 37°C for 30 minutes will aid dissolution of salt crystals.

Sample Handling and Storage

Collagen IV precipitates out of urine upon standing, leading to falsely low results. This can be prevented by the addition of a stabilizing buffer to the urine after which the urine can be stored. The Urinary Collagen IV Sample collection tubes provide a simple and reproducible means of collecting urine samples for the collagen IV assay.

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 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 collection tubes, samples can be stored at 4°C for one week or nine months at -20°C. If samples have been frozen, it is essential to mix thoroughly to dissolve any precipitates. Repeated freezing/thawing of samples should be avoided.

Samples and calibrators should be tested in duplicate. All solutions should be allowed to reach room temperature before use.

ASSAY PROCEDURE

NOTE: All reagents should be allowed to reach room temperature (RT = 20 - 27°C) prior to commencement of assay.

1. Immunoreaction

1.1 Prepare Wash Solution as described in “Preparation of Reagents”.
1.2. Place required number of Microwells in the assay plate (10 for the calibrator plus two for each sample).
1.3 Add 150 µL Conjugate to each well using a multi-channel 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 Microwell plate.
1.5 Cover the Microwell Plate with the Plate Seal and incubate at RT for 24 hours.
1.6 Remove the Plate Seal 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. Color development

2.1 Add substrate, 100 µL/well, using a multi-channel pipette and incubate at RT for exactly 1 hour.

3. Stop

3.1 Add Stop Solution 100 µL/well using a multi-channel pipette. Ensure complete mixing of Substrate and Stop Solution.
3.2 Read **immediately** at 450 nm using 630 nm as reference (if available).

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each Calibrator and sample.
2. Plot a calibration curve of $A_{450/630\ nm}$ versus collagen IV concentration (µg/L) on a log-log scale.
3. Read the collagen IV concentration (µg/L) indicated by the mean absorbance values of the samples from the calibration curve.
4. If the sample has been diluted, multiply the calculated collagen IV concentration by the appropriate dilution factor in order to obtain the actual collagen IV concentration.

PERFORMANCE CHARACTERISTICS

Limit of detection

The detection limit of the **K-*ASSAY*®** Human Collagen IV ELISA, Urine 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 **K-*ASSAY*®** Human Collagen IV ELISA, Urine 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.

Interference

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

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

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

Sample	Mean Collagen IV Conc., 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

Sample	Mean Collagen IV Conc., µg/L	SD	%CV	N
Low	2.4	0.05	2.1	4
Medium	6.0	0.15	2.5	4
High	20.8	1.52	7.3	4

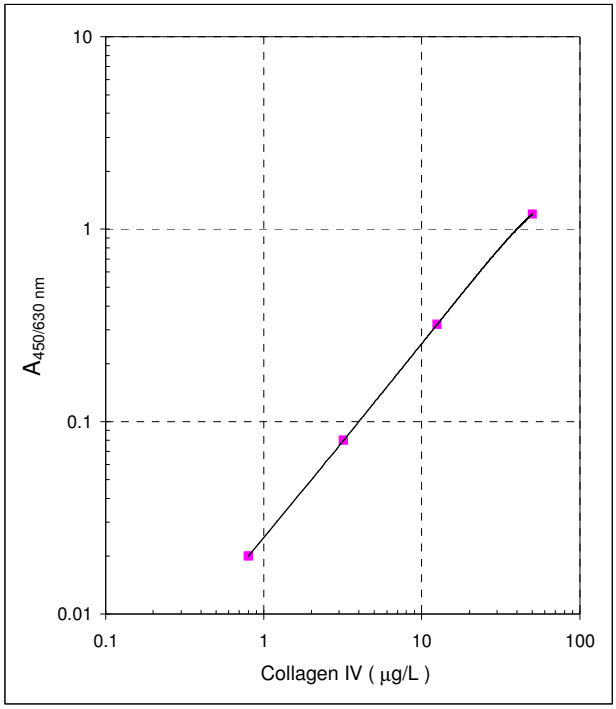
Inter-lot

Sample	Mean Collagen IV Conc., 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 A CALIBRATION CURVE

This curve is only an example. Each user must make their own calibration curve.

Figure 1: Typical calibration curve obtained using the **K-*ASSAY*®** Human Collagen IV ELISA, Urine.



STORAGE

1. All kit reagents should be stored at 4°C and are stable as supplied until the expiration date shown on the box label.
2. Prepared Wash Solution (PBT) is stable for up to one month at 4°C.
3. Microassay Wells should be stored in sealed bags with desiccant at 4°C until required for use. Return unused wells to the storage bag together with desiccant.
4. Urinary Collagen IV sample collection tubes are stable at RT until the expiration date shown on the box label. Return unused tubes to the storage bag.

WARNINGS AND PRECAUTIONS

Safety

- The **K-*ASSAY*®** Human Collagen IV ELISA, Urine is for *in vitro* research use only. Not for use in diagnostic procedures.
- The **K-*ASSAY*®** Human Collagen IV ELISA, Urine 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 sulfuric acid which is corrosive. Avoid contact with the skin or 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 samples and 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 samples and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by mouth and never eat or drink at the laboratory workbench.

Procedural

- Do not use the kit or individual reagents past their expiration 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 provided 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 de-ionized water is required for Wash Solution. The use of poor quality or contaminated water may lead to background color in the assay.
- Allow all reagents to come to RT and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 4°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.

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