KAMIYA BIOMEDICAL COMPANY

Human Collagen IV ELISA, Serum

For the quantitative determination of human Type IV Collagen in serum

Cat. No. KT-035

For Research Use Only. Not for Use in Diagnostic Procedures in the U.S.

PRODUCT INFORMATION

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

PRINCIPLE
The **K-ASSAY®** Human Collagen IV ELISA, Serum is designed for the assay of serum 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 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 enzyme-labelled antibodies. After removing unbound enzyme-labelled antibody and sample, the plate is incubated with enzyme substrate. The resultant color development is directly proportional to the amount of collagen IV in the sample.

COMPONENTS
1. Antibody coated Microtiter Plate: 12 x 8-well strips coated with IgG directed against human collagen IV. READY TO USE.
2. Collagen IV Calibrator: Purified collagen IV in phosphate buffer (pH 7.0) with protein stabilizer. 1,000 µg/L stock solution (1 mL). Contains 0.015% Geneticin as preservative. STOCK.
3. Dilution Buffer: Phosphate buffer (pH 7.0) containing protein stabilizer (5 mL). Contains 30 mg/L Proclin 300 as 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 Solution Concentrate: 10X Concentrated phosphate buffer with Tween 20 (2 bottles of 50 mL). Contains 30 mg/L Proclin 300 as preservative. CONCENTRATE.
6. 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: 1 sheet
9. Uncoated Microtiter Plate

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED
- Microtiter plate reader capable of measuring at 450 nm with reference at 630 nm if available.
- Precision pipettes 20 µL and 150 µL and a multi-channel pipette 100-150 µL with disposable tips.
- Microtiter plate washing system.
- 1 L beaker
- Timer
- Liquid trough
- De-ionized / Distilled water
- Graduated cylinder (500 mL)

PROTOCOLS
Preparation of Reagents
Wash Solution
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 10 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.

Calibrators
Using labelled tubes prepare Calibrators as follows:

Collagen IV Calibrators (A-H) Final Concentration (µg/L)		Add Calibrator (µL)	With Dilution Buffer (µL)
1,000	(A)	150 (A) (Stock Solution)	-----
500	(B)	150 (A) (Stock Solution)	150
250	(C)	150 (B)	150
125	(D)	150 (C)	150
62.5	(E)	150 (D)	150
31.2	(F)	150 (E)	150
15.6	(G)	150 (F)	150
0	(H)	-----	150

Calibrators should be prepared immediately before use. Do not store. The diluted Calibrators are stable for at least 6 hours at 4°C.

Sample Handling and Storage

Samples can be stored at 4°C for one week. Samples may be stored at -20°C for 12 months. Repeated freezing / thawing of samples should be avoided.

ASSAY PROCEDURE

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

1. Mixing of calibrator / sample

1.1 Prepare Wash Solution and Calibrators as described in "Preparation of Reagents".
1.2 Add Calibrators (H-A: 0-1,000 µg/L) and samples (20 µL/well) in duplicate to the Uncoated Microtiter Plate.
1.3 Add 150 µL Conjugate to each well.

2. Immunoreaction

2.1 Place required number of Anti-collagen IV coated microwells in the assay plate (16 for the Calibrators plus two each for the samples).
2.2 Transfer 100 µL of the mixtures from step 1 ("Mixing of calibrator / sample") into the equivalent wells in the Anti-collagen IV coated microwells.
2.3 Cover the Microtiter Plate with a Plate Lid and incubate at RT for exactly 30 minutes.
2.4 Remove the Plate Lid and wash each strip three 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.

3. Color development

3.1 Add Substrate, 100 µL/well, using a multi-channel pipette and incubate at RT for exactly 30 minutes.

4. Stop

4.1 Add Stop Solution, 100 µL/well, using a multi-channel pipette. Ensure complete mixing of Chromogenic Substrate and Stop Solution.
4.2 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 (15.6 to 1,000 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.
5. Concentrations of samples with readings outside the calibration curve are invalid and must be repeated with a higher dilution factor. It is not acceptable to extrapolate data.

PERFORMANCE CHARACTERISTICS

Limit of Detection

The detection limit of the ***K-ASSAY®*** Human Collagen IV ELISA, Serum is 15.6 µg/L.

Measuring Range
The calibration curve range covers the range 15.6–1,000 µg/L. The range may be extended by increasing sample dilution.

Specificity
The ***K-ASSAY®*** Human Collagen IV ELISA, Serum 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.

Sensitivity
When reading from the calibration curve the A_{450nm} value of the 1,000 µg/L calibrator should be >0.6 OD.

Interference
No significant interference has been observed in this assay with lipemic, hemolytic or icteric samples.
- Lipemia: Less than 10% interference up to 1,200 formazine turbidity units in the sample.
- Hemolysis: Less than 10% interference up to 3 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	57	61	107	28	32	114	14	16	114
B	107	110	103	53	58	109	27	28	104
C	259	270	104	130	139	107	65	67	103

Reproducibility

Intra-assay

Sample	Mean Collagen IV Conc., µg/L	SD	%CV	N
Low	119	7.4	6.2	8
Medium	218	7.8	3.6	8
High	520	12.0	2.3	8

Inter-assay

Sample	Mean Collagen IV Conc., µg/L	SD	%CV	N
Low	115	11	9.6	6
Medium	291	13	4.5	6
High	370	31	8.2	6

Inter-lot

Sample	Mean Collagen IV Conc., µg/L	SD	%CV	N
Low	115	5	4.3	3
Medium	270	6	2.2	3
High	386	16	4.1	3

Example of Calibration Curve

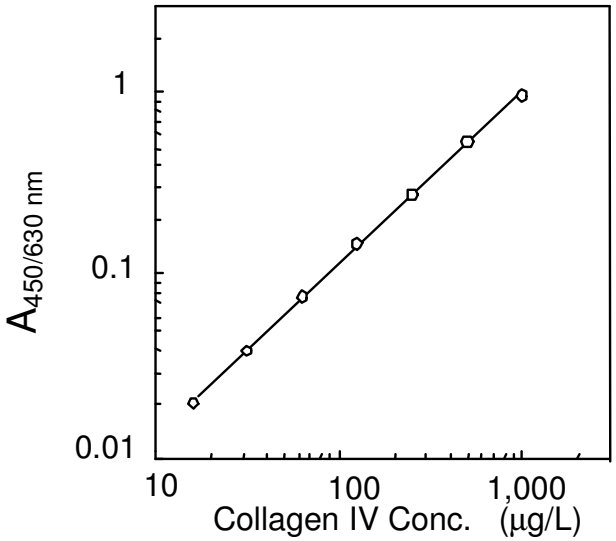

Figure 1: Typical calibration curve obtained using the ***K-ASSAY®*** Human Collagen IV ELISA, Serum. Assay range is 15.6-1,000 µg/L. Do not use this graph for calculations. The user must make a calibration curve for each assay.

STORAGE

1. All kit reagents should be stored at 4°C and are stable as supplied until the expiration date shown on the label.
2. Prepared Wash Solution is stable for up to one month at 4°C.
3. Prepared Calibrator Solutions should not be stored.
4. Microtiter Plate strips 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.

WARNINGS AND PRECAUTIONS

Safety

- The ***K-ASSAY®*** Human Collagen IV ELISA, Serum is for *in vitro* research use only, not for diagnostic procedures.
- The ***K-ASSAY®*** Human Collagen IV ELISA, Serum 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 that 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 diluting 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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