TECOmedical Group

TECO®

Serum Collagen IV ELISA

Kit instruction
English

Catalogue No. TE 1053
For Research Use Only

TE1053 | 05/2017 © **TECO***medical* Group | 07/2017 **DiaPharma** Group TE1053Rev01

Symbol Description

Kit Instructions

Lot Number

Expiry Date

Storage Temperature

Manufacturer

TE 1053

Caution: caustic

Intended use

Tests

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TECO® Serum Collagen IV ELISA Kit

CONT **Reagents and Materials Supplied:**

SYMBOL	DESCRIPTION	FORMAT
1	**Collagen IV Antibody coated Microassay Plate** 12 strips of 8 wells (96 break apart wells in total), in a frame. Ready to use.	**1 Plate**
S	**Collagen IV Calibrator** Stock Solution, 1000 µg/L	**1 x 1.0 ml**
2	**Wash Buffer: 10x concentrate**	**1x 50 ml**
3	**Dilution Buffer** Ready to use.	**1 x 5 ml**
6	**Enzym Conjugate: Anti-collagen IV mouse Fab' conjugated to horseradish peroxidase** Ready to use.	**1 x 20 ml**
7	**TMB Substrate** Ready to use.	**1 x 15 ml**
8	**Stop Solution: 1M Sulphuric Acid** Ready to use.	**1 x 15 ml**
9	**Uncoated Microassay Plate** Ready to use.	**1 Plate**
	Kit instructions	**1 x**

Storage
Store kit at 2–8 °C. Do not freeze. Store unused reagents at 2–8 °C.

INTENDED USE

The TECO®Serum Collagen IV ELISA provides a method for the quantitative determination of collagen IV in human serum. Please contact TECOmedical for further information regarding the assay of collagen IV in other tissue fluids. The TECO®Serum Collagen IV ELISA is for research use only. Not for use in diagnostic procedures.

BACKGROUND

SERUM

Chronic liver disease comprises a number of progressive disorders which culminate in liver cirrhosis and which are characterized by excessive deposition of collagen. Although various types of collagen (type I, III, IV, V and VI) increase in the liver with the progression of fibrosis, type IV collagen, a constituent of the basement membrane, is particularly noteworthy for the following reasons: its serum level correlates with hepatic levels of collagen IV[1], serum levels of collagen IV fall in response to effective therapy1 and it is the earliest type of collagen to be synthesized during experimental liver injury[2,3]. Serum collagen IV levels are elevated in a variety of liver diseases[4-6], in particular, serum collagen levels have been found to be predictive of therapy response in Hepatitis C infection[7], and to be sensitive indicators of therapy response in abstaining alcoholics[1].

REFERENCES

1) **Tsutsumi, M. *et al***.(1996). Serum biomarkers for hepatic fibrosis in alcoholic liver disease: which is the better biomarker, type III procollagen, type IV collagen, laminin, tissue inhibitor of metalloprotease or prolyl hydroxylase? Alcoholism: Clinical and Experimental Research. **20(9)**, 1512-1517.
2) **Gay, S. and Miller, E.J.** (1983). What is collagen, what is not. Ultrastruct. Pathol. **4,** 365-377.
3) **Dingleman, R.F. *et al***.(1983). Collagen formation by the hepatocyte in primary monolayer culture and *in vivo*. Science, **219,** 1343-1345.
4) **Obata, K. *et al*.** (1989). One step sandwich enzyme immunoassay for human type IV collagen using monoclonal antibodies. Clin. Chim. Acta, **181,** 293- 304.
5) **Yokoya, Y. *et al.*** (1992). Concentration of serum laminin and type IV collagen in liver diseases assayed by a sandwich enzyme-immunoassay using monoclonal antibodies. Clin. Chim. Acta, **210**, 109- 118,
6) **Ueno, T. *et al***.(1992). Significance of serum type-IV collagen levels in various liver diseases. Scandinavian Journal of Gastroenterology **27**, 513-520.
7) **Yabu, K. *et al.*** (1994). Serum collagen IV for the assessment of fibrosis and resistance to Interferon therapy in chronic hepatitis C. Scandinavian Journal of Gastroenterology. **29**, 474-479.

ASSAY PRINCIPLE

The TECO®Serum Collagen IV ELISA 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.

Materials Required and not Supplied

- Pipettes 20 µL – 150 µL
- Multichannel pipettes for 100-150 µL
- Graduated cylinders for reconstituting or diluting reagents
- Manual Aspiration System or Automatic washer for ELISA plates
- Aqua dest.
- Vortex mixer
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 nm (Reference: 590-650 nm)
- ELISA plate shaker (500 rpm) (orbital shaker)
- Software package for data generation and analysis

Warnings and Precautions

This kit is intended for research use by professional persons only.
Follow the instructions carefully.

Observe expiration dates stated on the labels and the specified stability for reconstituted reagents. Refer to ”Materials Safety Data Sheet” for more detailed safety information.

Material of animal origin used in the preparation of this kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious.

Material of human origin used in the preparation of this kit has been tested and found non reactive for HIV-1 and HIV-2 as well as for HCV antibodies and HbsAg but should, nonetheless, be handled as potentially infectious.

TECOmedical AG is not liable for loss or harm caused by non-observance of the Kit instructions.

1 For research use only.
2 Treat all specimen samples as potentially biohazardous material.
Follow General Precautions when handling contents of this kit and any patient samples.
3 Disposal of containers and unused contents should be done in accordance with federal and localregulatory requirements.
4 Use the supplied reagents as an integral unit prior to the expiration date indicated on the package label.
5 Store assay reagents as indicated.
6 Do not use coated strips if pouch is punctured.
7 Test each sample in duplicate.
8 Use of multichannel pipettes is recommended to ensure the timely delivery of liquids.
9 a. 1 M sulphuric acid is caustic and can be harmfull for skin, eyes and mucosae.
b. Handle TMB with care. Do not ingest. Avoid contact with skin, eyes, or clothing. Should there be any contact, wash with water. If ingested, call a physician.
10 A mercury-free preservative is used. Incidental contact with or ingestion of buffer solutions may cause irritation of skin, eyes or mouth. Should there be any contact, wash with water. If ingested, call a physician.

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 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. Prepared Calibrator solutions should not be stored.
4. Plate assay wells should be stored in sealed bags with dessicant at 2-8°C until required for use. Return unused wells to the storage bag together with dessicant.

PREPARATION OF REAGENTS

WASH SOLUTION (PBT)

Perform a 1/10 dilution of Wash Concentrate adding, for example, 10 mL 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 10 mL of Wash 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.

CALIBRATORS

Using labelled tubes prepare Calibrators as follows:

Collagen IV Concentration (µg/L)	Calibrator Volume (µL)	Dilution Buffer (µL)
1000 (A)	150 (A) (Stock Solution)	-
500 (B)	150 (A)	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 2-8° C.

Sample Handling and Storage

Samples can be stored at 2-8°C for one week. Samples may be stored at –20°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. MIXING OF CALIBRATOR/SAMPLE

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

1.2 Add Calibrators **(H-A: 0 – 1000 µg/L)** and samples **(20 µL/well)**, in duplicate, to the uncoated Vinyl Microassay plate.

1.3 Add **150 µL** Conjugate to each well.

2 IMMUNOREACTION

2.1 Place required number of anti-collagen IV coated Microassay wells in the assay plate (16 for the Calibrators plus two each for the samples).

2.2 Transfer **100 µL** of the mixtures from above into the equivalent wells in the anti-Collagen IV coated Microassay wells.

2.3 Cover the Microassay plate with the lid and incubate at (20-27°C) for exactly **30 minutes.**

2.4 Remove the plate lid and wash each strip three times **(350 µL/well)** with 1x Wash Solution. When complete, firmly tap the plate against a paper towel to ensure complete removal of wash fluid from wells.

3. COLOUR DEVELOPMENT

3.1 Add **100 µL** Substrate/well using a multichannel pipette and incubate at (20-27^{o}C) for exactly **30 minutes**.

4. STOP

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

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

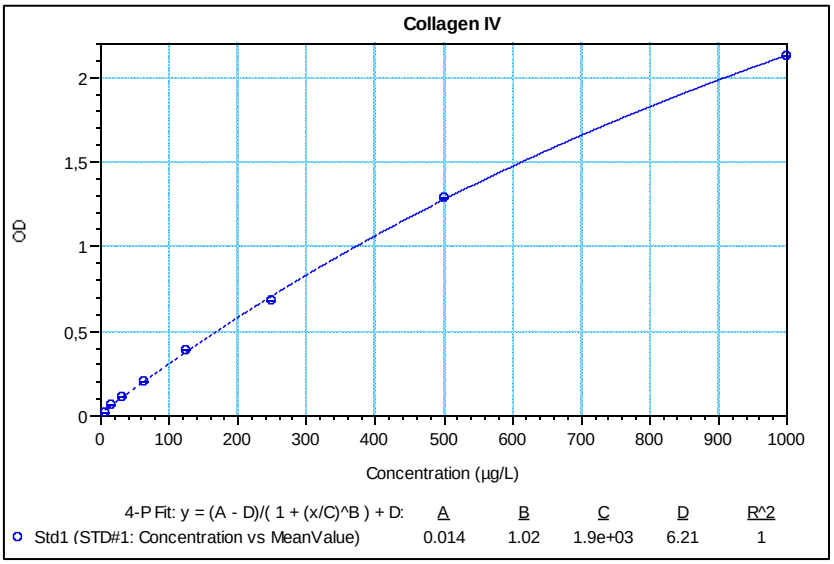
CALCULATION OF RESULTS

1. Calculate the mean absorbance for each calibrator and sample.
2. Plot a Calibration curve of $A_{450/630nm}$ 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].
5. Concentrations of samples with readings outside the standard curve are invalid and must be repeated with a higher dilution factor. It is not acceptable to extrapolate data.

Example of Calibration Curve

(Example only, not for use in calculation of actual results)

Standard	Concentration (µg/L)	OD (450 nm)
A	1000	2.127
B	500	1.292
C	250	0.677
D	125	0.389
E	62.5	0.205
F	31.3	0.113
G	15.6	0.065
H	0	0.021



PERFORMANCE CHARACTERISTICS

NORMAL RANGE

Based on healthy Japanese volunteers, the reference normal range for Collagen IV is: 99 ± 23 µg/L. Mean ± 1S.D. (N = 180).

LIMIT OF DETECTION

The detection limit of the TECO®Serum Collagen IV ELISA is 15.6 µg/L.

MEASURING RANGE

The calibration curve range covers the range 15.6-1000 µg/L. This range may be extended by increasing sample dilution.

SPECIFICITY

The TECO®Serum Collagen IV ELISA 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 standard curve the A_{450nm} value of the 1000 µg/L standard should be >0.6 OD.

INTERFERENCE

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

- Lipaemia: Less than 10% interference up to 1200 Formazine turbidity units.
- Haemolysis: Less than 10% interference up to 3 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	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 variation of the TECO®Serum Collagen IV ELISA.

Sample	$\bar{X}$ [Collagen IV] µg/L	SD	%CV	N
Low	119	7.4	6.2	8
Medium	218	7.8	3.6	8
High	520	12	2.3	8

Inter-assay variation TECO®Serum Collagen IV ELISA.

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

Inter-batch Variation of the TECO®Serum Collagen IV ELISA calculated for three batches of kits.

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

NOTE:

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TECO®Serum Collagen IV ELISA

Assay Procedure – Quick Guide

- Bring samples and reagents to 20-27°C. Mix the samples well.
- Dilute Wash Buffer 2 1:10 with deionized or destilled water.
- Prepare standards as described in 'Preparation of Reagents'.
- Prepare the required number of Assay strips 1.

Add **20 µL/well** calibrators **(H-A)** and samples, in duplicate, to the uncoated Vinyl Microassay plate 9.

Add **150 µL** Conjugate 6 to each well

Place required number of anti-collagen IV coated Microassay wells 1 in the assay plate (16 for the Calibrators plus two each for the samples).

Transfer **100µL** mixture in the anti-Collagen IV coated Microassay wells.

Incubate at 20-27°C for exactly **30 minutes.**

Wash each strip three time **(350 µL/well)** with 1x Wash Solution.

Add **100 µL** Substrate/well 7.

Incubate at 20-27°C for exactly **30 minutes.**

Add **100 µL** Stop Solution/well 8.
Ensure complete mixing of Substrate and Stop Solution.

Read **immediately** at 450 nm using 630 nm as reference.
Analyze the assay results using a 4-parameter curve fit: y=(A-D)/(1+(x/C)B)+D

Please read the Kit Instructions before using the Quick Guide.