Product Manual

Human Collagen Type III (COL3) ELISA Kit

Catalog No.: abx252253

Size: 96T

Range: 0.625 - 40 ng/ml

Sensitivity: < 0.375 ng/ml

Storage: Store at 4°C for up to 6 months

Application: For quantitative detection of Collagen Type III (COL3) in Human serum, plasma, tissue homogenates and other biological fluids.

Introduction

Collagen is the main structural protein in the extracellular space in the various connective tissues in animal bodies. As the main component of connective tissue, it is the most abundant protein in mammals, making up from 25% to 35% of the whole-body protein content. Depending upon the degree of mineralization, collagen tissues may be rigid (bone), compliant (tendon), or have a gradient from rigid to compliant (cartilage).

Principle of the Assay

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. COL3 antibody is pre-coated onto 96-well plates. Biotin conjugated antibody is used as a detection antibody. The standards, test samples and detection antibody are added to the wells and washed with wash buffer. HRP Streptavidin is added and unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue colour product that changes into yellow after adding stop solution. The density of yellow is proportional to the COL3 amount of sample captured in plate. The O.D. absorbance is measured spectrophotometrically at 450nm in a microplate reader, and then the concentration of COL3 can be calculated.

Kit Components

1. One pre-coated 96 well plate
2. Standard: 2 tubes
3. Sample/Standard diluent buffer: 20ml
4. Biotin conjugated antibody (Concentrated): 120µl, Dilution 1:100
5. Antibody diluent buffer: 10ml
6. HRP streptavidin conjugate (SABC) (Concentrated): 120µl, Dilution 1:100
7. SABC diluent buffer: 10ml
8. TMB substrate: 10ml
9. Stop solution: 10ml
10. Wash buffer (25X): 30ml

Material Required But Not Provided

1. 37°C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions
7. Absorbent filter papers
8. 100 ml and 1 L volume graduated cylinder.

Protocol

A. Preparation of sample and reagents

1. Sample

Store samples to be assayed within 24 hours at 2-8°C. Alternatively, aliquot and store at -20°C or -80°C for long term. Avoid repeated freeze-thaw cycles.

✧ **Tissue homogenates:** The preparation of tissue homogenates will vary depending upon tissue type – this is just an example. Rinse tissues with ice-cold PBS (0.01 mol/L, ph 7.2) to remove the excess of blood. Weigh before homogenization. Finely mince tissues and homogenize with a tissue homogenizer on ice in PBS and sonicate the cell suspension. Centrifuge the homogenates at 5000 × g for 5 min and collect the supernatant. Assay immediately or aliquot and store at -20°C.

✧ **Serum:** Allow the serum to clot in a serum separator tube at room temperature (2 hours). Centrifuge at approximately 1000 × g for 20 minutes. Analyze the supernatant immediately or aliquot and store at -20°C.

✧ **Plasma** Collect plasma using EDTA-Na2 as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.

✧ **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results. It is recommended to store samples to be used within 5 days at 4°C, within 1 month at -20°C and within 2 months at -80°C.
- Samples should be clear and transparent. Hemolyzed samples are not suitable for use in this assay.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.

General Sample guideline:

Estimate the concentration of the target in the sample and select the correct dilution factor to make the diluted target concentration fall near the middle of the kit's range. Generally, for high concentration, dilute 1:100, for medium concentration, dilute 1:10 and for low concentration, dilute 1:2. Very low concentrations do not need dilution. Dilute the sample with the provided Sample Diluent Buffer and mix thoroughly. Several trials may be necessary to determine the optimal dilution factor.

2. Wash buffer

Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water).

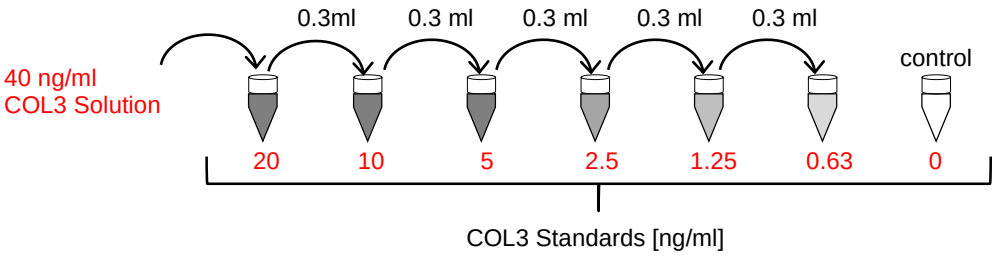
3. Standard

Preparation of the COL3 standard: standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of standard are included in each kit. Use one tube for each experiment. **(Note: Do not dilute the standard directly in the plate).**

a.) 40 ng/ml standard solution. Add **1 ml** of Sample/Standard diluent buffer into one Standard tube, keep the tube at room temperature for 10 min, mix thoroughly and avoid foaming or bubbles.

b.) 20 ng/ml → 0.63 ng/ml standard solutions: Label 6 tubes with 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml

and 0.63 ng/ml. Aliquot **0.3 ml** of the Sample / Standard diluent buffer into each tube. Add **0.3 ml** of the above 40 ng/ml standard solution into 1st tube and mix thoroughly. Transfer **0.3 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on.



Note: The standard solutions are best used within 2 hours. The standard solution can be stored at 4°C for up to 12 hours, or at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

4. Preparation of Biotin conjugated antibody working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the Biotin conjugated antibody with Antibody diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of Biotin conjugated antibody into 99 µl of Antibody diluent buffer.

5. Preparation of HRP Streptavidin Conjugate (SABC) working solution: prepare no more than 1 hour before the experiment.

a.) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume).

b.) Dilute the SABC with SABC diluent buffer at 1/100 and mix thoroughly. i.e. Add 1 µl of SABC into 99 µl of SABC diluent buffer.

B. Assay procedure

Equilibrate the SABC working solution and TMB substrate for at least 30 minutes to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. **Wash the plate two times before adding standard, samples and buffers.** Any strips that are not being used should be kept dry and stored at 4°C. Set standard, test sample and control (zero) wells on the pre-coated plate and record their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 100 µl of the prepared standards solutions into the standard wells.
3. Add 100 µl of Sample / Standard diluent buffer into the control (zero) well.
4. Add 100 µl of appropriately diluted sample into test sample wells.
5. Cover the plate and incubate at 37°C for 90 minutes.
6. Remove the cover and discard the contents by clapping the plate on absorbent filter papers or any other absorbent material. Do not wash the plate and do NOT let the wells dry out completely at any time.
7. Add 100 µl of Biotin conjugated antibody into each well (standard, test sample and zero well). Add the solution at the bottom of each well without touching the side walls. Seal the plate with a cover and incubate at 37°C for 60 minutes.

8. Remove the cover, and wash the plate 3 times. Discard the solution without touching the side walls. Blot the plate on an absorbent material. Fill each well completely with wash buffer and soak for at least 1-2 min. Discard the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of three times.

   Please note: For automated washing, discard the solution in all wells and wash three times overfilling the wells with Wash buffer. After the final wash invert the plate and tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

9. Add 100 µl of SABC working solution into each well, cover the plate and incubate at 37°C for 30 minutes.

10. Remove the cover and wash the plate 5 times with Wash buffer. Allow the wash buffer to remain in the wells 1-2 min for each wash. Discard the washing buffer and blot the plate onto absorbent filter papers or other absorbent material.

11. Add 90 µl of TMB substrate into each well. Cover the plate and incubate at 37°C in dark conditions for 15-30 minutes. The incubation time is for reference only, the optimal time should be determined by the end user. Please do not exceed 30 min.

12. Add 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.

13. Read the O.D. absorbance at 450 nm in a microplate reader within 30' of adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human COL3 concentration of the samples can be extrapolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from extrapolation to obtain the concentration before dilution.

C. Precautions

1. Ensure that the plate remains dry until starting the assay.
2. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. Avoid foaming or bubbles when mixing or reconstituting components.
5. It is recommended to assay all standards, controls and samples in duplicate or triplicate.
6. Do NOT let the plate dry out completely during the assay as this will inactivate the biological material on the plate.
7. To avoid cross contamination do not reuse pipette tips and tubes.
8. Do not use components from a different kit or expired ones.
9. The TMB substrate is light sensitive and should be protected from direct sunlight and UV sources. Unreacted substrate should be colorless or very light yellow in appearance. The product should be allowed to equilibrate to room temperature (25°C) prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Typical Data & Standard Curve

Typical Standard Curve Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

ng/ml	0	0.63	1.25	2.5	5	10	20	40
OD450	0.035	0.106	0.174	0.313	0.520	0.945	1.698	2.465