Human Collagen, type I, Alpha 1 (COL1A1) ELISA Kit

96 Well

Catalogue Number: NE01C1899

Valid Period: 6 months

For samples:

Serum, plasma, and tissue homogenates

FOR RESEARCH USE ONLY!

NOT FOR THERAPEUTIC OR DIAGNOSTIC APPLICATIONS!

PLEASE READ THROUGH ENTIRE PROCEDURE BEFORE BEGINNING!

CONTENTS

1. APPLICATION

This Human COL1A1 ELISA kit is for research use only, it should not be used in clinical diagnostic procedures.

2. BACKGROUND

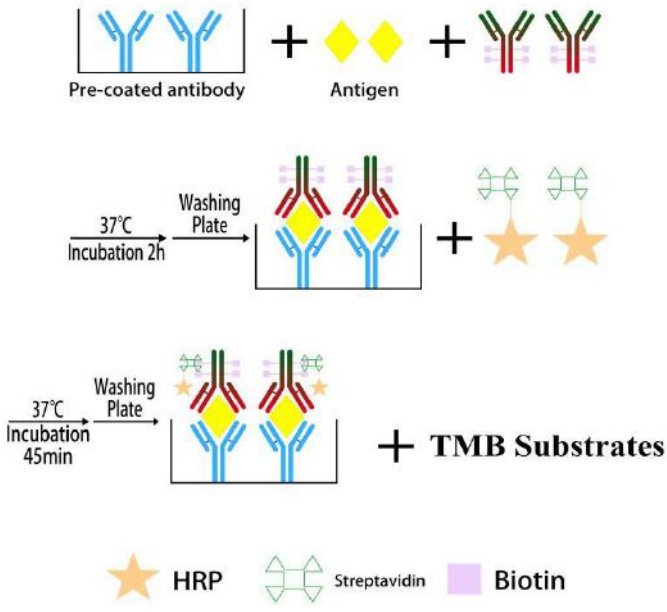
Type I collagen is a structural protein that is abundant in skin, bone and other tissues. It is synthesized as a procollagen molecule which is shown as a 300 nm triple helical domain flanked by globular N- and C-terminal propeptides. When the non-helical propeptides are removed by procollagen N-terminal and C-terminal proteinase activities, the mature triple helices are able to self-assemble into collagen fibrils that enhance tensile strength to tissues.

Type I collagen is a heterotrimer in structure, which consists of two alpha 1 chains and one alpha 2 chain. As a heterotrimer in structure, although Type I collagen is also described as homotrimers consisting of three identical alpha 1 chains. Mutations in this gene are associated with osteogenesis imperfecta types I-IV, Ehlers-Danlos syndrome type VIIA, Ehlers-Danlos syndrome Classical type, Caffey Disease and idiopathic osteoporosis. Reciprocal translocations between chromosomes 17 and 22, where this gene and the gene for platelet-derived growth factor beta are located, are associated with a particular type of skin tumor called dermatofibrosarcoma protuberans, resulting from unregulated expression of the growth factor. Two transcripts, resulting from the use of alternate polyadenylation signals, have been identified for this gene.

3. PRINCIPLE OF THE ASSAY

This ELISA kit is applied to the solid phase sandwich Enzyme Linked Immunosorbent Assay. The microtiter plate has been pre-coated with capture antibody. The antigen to be measured and the biotin-conjugated detection antibody are then added to the microtiter plate wells sequentially to form a capture antibody-antigen-detection antibody complex. After washing, Streptavidin conjugated Horseradish Peroxidase (HRP) is added to the wells, and then a TMB substrate solution is added to the wells to incubate. TMB substrate solution turns blue after the oxidation of HRP, and finally turns to yellow immediately after adding the stop solution. Color of TMB substrate positively correlated with total COL1A1 bound in the initial steps. The color is measured by spectrophotometrically with

wavelength of 450nm. The concentration of COL1A1 in samples is then determined by comparing the O.D. of the samples to the standard curve.



ELISA PRINCIPLE FLOWCHART

4. KITS' ADVANCEMENT

1) High Specificity: Capture antibody and detection antibody respectively identify different epitopes of the antigen, which maximizes the specificity of the reaction. No cross-reaction exists between the homologous cytokines.
2) High Sensitivity: Detection antibody conjugated biotin cross-link with polyvalent and high-affinity avidin to initiate cascade amplification to maximize the sensitivity.
3) High Stability: the experiment uses high-quality coated antibodies and antigens, and also applies the broad spectrum protein stabilizers, and antibody pre-coated microplate treatment to increase the thermostability of the microplate, and reproducibility of results.
4) Optimal Diluent Buffer: Use a specific buffer optimized for human serum samples to get rid of matrix interference. It is suitable for quantification of cytokines in serum, and plasma.

5. MATERIALS (Note: Storage at 2-8℃)

	REAGENTS	SPECIFICATION	QUANTITY

1	Pre-Coated Microplate (Detachable)	96 wells	1 plate (Keep Sealed)
2	Standard (Stock Solution-14ng/ml)	200ul	1 vial
3	Standard Diluent S1	900ul	1 vial
4	Standard Diluent S2	500ul	1 vial
5	Standard Diluent S3	500ul	1 vial
6	Standard Diluent S4	500ul	1 vial
7	Standard Diluent S5	500ul	1 vial
8	Standard Diluent S6	500ul	1 vial
9	Standard Diluent S7	500ul	1 vial
10	Standard Diluent S8	500ul	1 vial
11	Detection antibody (15×)	450ul	1 vial
12	Detection antibody Diluent	6ml	1 vial
13	HRP-Streptavidin (150×)	100ul	1 vial
14	HRP-Streptavidin Diluent	10.5ml	1 vial
15	TMB Substrates	11ml	1 vial (Avoid Light)
16	Stop Solution	6ml	1 vial
17	Wash Solution (100×)	10ml	1 vial
18	Plate Sealer		4 pieces
19	Instruction Manual		1

6. EQUIPMENT REQUIRED BUT NOT PROVIDED

1) Precision pipettors and disposable tips to deliver 10-1000 μl.
2) A multi-channel pipette is desirable for large assays.
3) 1L sterilized deionized water or hyperpure water.
4) Sterilized EP Tubes

5) Absorbent Paper

6) Microplate reader capable of measuring absorbance at 450 nm.

7) High-speed Centrifuge

8) Automated Microplate Washer and Washing Bottle.

9) Incubator or Water bath (37°C).

10) Data analysis and graphing software.

11) Preparation of PBS: NaH2PO4 0.2g, Na2HPO4·12H2O 2.9g, NaCl 8g，KCl 0.2g. Then dilute these to 1000ml with distilled water

7. SPECIMEN COLLECTION AND STORAGE

Serum - Collect blood sample in a serum isolation tube and allow the sample to clot for 1- 2 hours at room temperature. Centrifuge at 1000 × g (or 3000 rpm) for 15 minutes. Remove serum for immediate assay or aliquot and store serum sample at -20°C or -80°C.

Plasma – Add 1% total sample volume of EDTA or heparin etc. as an anticoagulant to collect plasma. Allow samples to stand for 30 mins at room temperature or 4℃. Mix, then centrifuge samples for 15 minutes at 1000 × g (or 3000 rpm). Remove plasma for immediate assay or aliquot and store samples at -20°C or -80°C.

Tissue homogenate -Preparation of tissue homogenate depends upon tissue type. For this assay, rinse tissues in ice-cold PBS (0.02mol/L, pH 7.0-7.2), remove excess blood thoroughly, and collect 300-500mg of tissue before homogenization. Mince tissue to small pieces and homogenize in 500ul of PBS with a glass homogenizer on ice. The resultant suspension is subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membrane. After centrifugating for 15 minutes at 1500 × g (or 5000 rpm),remove the supernatefor immediate assay or aliquot and store samples at -20°C or -80°C.

Cell lysates - Cells lysate preparation according to the following directions.

1) Collect suspension cells by centrifugation directly. Adherent cells need trypsin treatment, and then collect the detached cells by centrifugation (Certain types of cells can't be detached with trypsin).

2) Wash the cells three times in PBS (0.02mol/L, pH7.0-7.2).

3) Resuspend the cells in PBS for ultrasonication or to two freeze-thaw cycles to break the cell membrane further.

4) Centrifuge cells lysate for 15 minutes at 1500×g (or 5000 rpm).

5) Remove the supernatantfor immediate assay or aliquot and store samples at -20°C or -80°C.

Cell culture supernatant - Centrifuge cell culture supernatant at 1000×g (or 3000 rpm) for 15 minutes,and collect the supernatant for immediateassay or store samples at -20°C or -80°C.

NOTE:

- Samples should be aliquoted and must be stored at -20°C (less than 3 months) or -80°C (less than 6 months) to avoid loss of bioactivity and contamination. If samples are to be run within 24 hours, they may be stored at 2- 8°C. Avoid repeated freeze-thaw cycles. Fresh samples without long time storage are recomended for the test. Otherwise, protein degradation and denaturalization may occur in those samples.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Samples containing a visible precipitate must be clarified prior to use in the assay.
- Care should be taken to minimize hemolysis. Do not use grossly hemolyzed or lipemic specimens.
- Do not use heat-treated specimens.

8. PREPARATION BEFORE ASSAY

1) Please read through this manual carefully before using, and set the temperature at 37 °C for Incubator or Water bath.

2) The frozen samples should be slowly restored to room temperature and then mix well, centrifuge the samples at 12000 rpm for 1 minute. Collect the supernatant for immediate assay.

3) Sample Dilution: Please predict the concentration before assaying. If values for these are exceed the highest detection range of the kit, please use the PBS to make the dilutions.

NOTE:

- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Influenced by the factors including low cell viability, small cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
- Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples, and lead to inaccurate results.
- The optimal pH value of the sample is between 7.0-7.4.

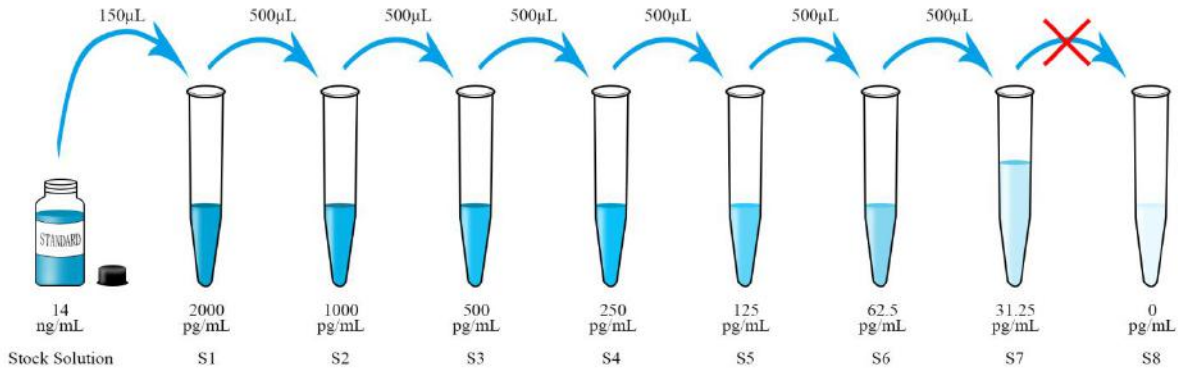
9. REAGENT PREPARATION

1) Place all kits' components at room temperature for 30mins before using.
2) Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized water to prepare 1000 mL of Wash Solution (1×). If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. The 1× wash solution is stable for 2 weeks at 2-8°C.

10. ASSAY PROCEDURE

1) Remove the microtiter plates from the foil bag, detach the desired number of strips from the plate, immediately reseal the remaining strips and store at 4° C. Then put the ready-for-use strips on a clean micropore plate frame, and start the experiment after the strips returned to room temperature.（Note: The microplate frame can be reused.）
2) Prepare Standards: The concentration of Standard (Stock Solution) is 14ng/ml. Adding 150ul of Standard (Stock Solution) into Standard Diluent S1, then produce a 2-fold dilution series (see below). Secure the desired plate in the holder then add 100ul of diluted standards or 100ul (diluted or undiluted) samples to appropriate wells.

   NOTE: There are 8 points of diluted standards, S1: 2000pg/ml, S2: 1000pg/ml, S3: 500pg/ml, S4: 250pg/ml, S5: 125pg/ml, S6: 62.5pg/ml, S7: 31.25pg/ml, S8: 0pg/ml.



3) Prepare Detection Antibody: Extract 400ul of detection antibody (15×), and add it into detection antibody diluent to reach its working concentration (1×) and mix gently.Add 50ul of above diluted detection antibody to each well, cover the plate with a sealer, and put it on the oscillator to mix and incubate for 120 mins at room temperature.

4) Automated Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle of the automated microplate washer for standby.
- Take out the microplate, and discard the incubation mixture. And then wash the microplate with the automated microplate washer for 5 times.
- After washing, invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.

Or Manual Washing:

- Put 1000 ml Wash Solution (1×) into the washing bottle for standby.
- Take out the microplate, and discard the incubation mixture. And then invert plate and blot dry by hitting the plate onto absorbent paper or paper towels until there is no moisture appears.
- Fill each well with 300ul Wash Solution (1×) by a muti-channel pipette, let stand for 20 seconds, then discard the contents, invert plate, and blot dry by hitting the plate onto absorbent paper. Repeat this washing step for 5 times. Note: There should be no moisture appears in the fifth washing step.

5) Prepare HRP-Streptavidin: Extract 70ul HRP-Streptavidin (150×), and add it into HRP-Streptavidin diluent to reach its working concentration (1×) and mix gently.Add 100ul of

above diluted HRP-Streptavidin to each well, cover the plate with a new sealer, and incubate for 45 mins at room temperature.

6) Washing Step: Repeat the same procedure as step 4.

7) Add 100ul TMB substrate to each well. Put it on the oscillator to mix for 30s. Cover the plate with a sealer, incubate at 37℃ for 10 to 30 mins. (Avoid sunlight). Note: Please set the speed of the oscillator at 100rpm or less. Liquid spills will effect the OD. The coloring time varies in different experimental conditions (temperature, humidity, ect.).

8) Add 50ul of Stop Solution to each well to stop the reaction. Put it on the oscillator to mix well for 30s or mix the liquid by gently tapping the side of the plate.

9) Run the microplate reader and conduct measurement at 450nm.

10) Data analysis: Four parameter curve fitting is recommended.

11. NOTES

1) **Sample Preparation**

a) After collection, the samples should be aliquoted and stored at -20℃ (less than 3 months) or -80℃ (less than 6 months) to maintain protein activity, avoid contamination and multiple freeze-thaw cycles. If samples are to be run within 24 hours, they may be stored at 2- 8°C.

b) Samples should be frozen and aliquoted if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well by pipette or Vortex, centrifuge to remove flocculent insoluble substances before use.

c) If turbidity appears in the stock solution (Standard, Detection antibody and HRP-Streptavidin), mix gently or pipette up and down for several times.

d) It is recommended that all standards, controls and samples be run in duplicate. Please gently mix the liquid after adding standards and samples, and confirm there's no bubble inside.

e) Do not use hyperlipidemia or hemolysis samples, which may interfere with ELISA and lead to inaccurate results.

2) Experimental Operation

a) Do not mix or interchange different ELISA kits or same ELISA kit but with different lot.

b) To avoid cross-contamination, change pipette tips between different diluted standard additions, sample additions, and reagent additions.

c) Total dispensing time for addition of reagents or samples to the plate should not exceed 10 minutes. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.

d) Incomplete washing will adversely affect the test outcome. All washing must be performed with Wash Solution provided. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into the wells. Do not directly touch the bottom of the microplate. If it is covered with fingerprints or stains, it will affect the measurement.

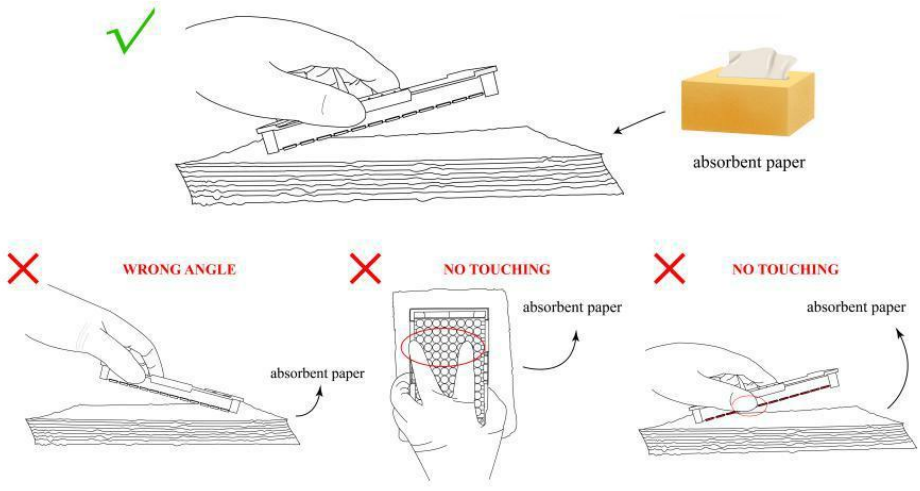
e) TMB is easily contaminated. Please protect it from light and metal.

f) Monitor the reaction time. After adding TMB substrate, observe the color change of reaction wells at regular intervals (for example, about 10 minutes). If the color turns too deep, add Stop Solution in advance to terminate the reaction.

g) The Stop Solution provided with this kit is acid solution. Protective gloves, clothes, and face protection should be worn.

h) The coefficient of determination of the standard curve should be $R^2 \geq 0.95$.

i) Schematic diagram of tapping the plate.

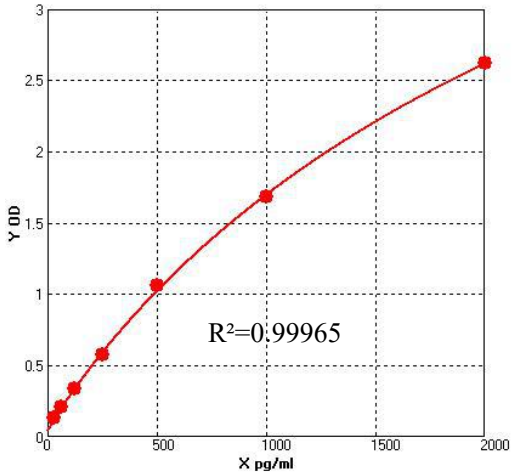


12. CALCULATION OF RESULTS

1) Average the duplicate readings for each standard and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
2) Construct a standard curve by plotting the average O.D. for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and generate a four paramater logistic (4-PL) curve-fit.
3) Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.
4) Standard curve is for demonstration only.

APPENDIX 1: EXAMPLE OF STANDARD CURVE

COL1A1 Standard curve



pg/ml	OD
2000.00	2.625
1000.00	1.686
500.00	1.060
250.00	0.577
125.00	0.337
62.50	0.208
31.25	0.132

13. QUALITY CONTROL

1) Intra Variation%: 5.1-5.9
2) Inter Variation%: 4.9-7.0
3) Recovery%: 80-111 (serum)
4) Linearity:

Diluent ratio	Range %
1:2	99.40-100.21
1:4	103.78-115.27
1:8	101.11-103.93

1:16	95.19-114.92
1:32	104.38-105.86
1:64	83.87-98.80

5) Sensitivity: 6.4pg/ml

6) Specificity/Cross-reactivity:

Sample	Cross reactivity（%）
IL-18	0.36
Ang-2	---
MMP-1	---
MMP-9	---

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