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USER MANUAL



ELISA KIT

Human Collagen Type I ELISA Kit (COL1)

Catalog No. tcbe113125 96 Tests

FOR RESEACH USE ONLY!

Please read completely user manual and storage condition.

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Human Collagen Type I ELISA Kit (COL1)

Product No. tcbe113125

Available Sizes

Size: 96 Tests

Specifications

Research Area:	Human Collagen
Species Reactivity:	Human
Sample Type:	Serum, plasma, Cell lysates, Cell culture supernatant, biological fluids
Sensitivity:	0.059 ng/mL were assayed and exhibited no cross-reactivity or interference.
Recovery:	78-101% with an average recovery
Detection Range:	62.5-4000 pg/mL
Assay Time:	4 hrs
Detection Method:	Competitive ELISA Colorimetric; absorbance at 450 nm
Precision:	Intra-assay coefficient of variation (CV) <10%. Inter-assay CV <10%. Same Lot CV (5.6%)

Introduction

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of COL1 in human s erum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Test Principle

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for COL1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any COL1 present is bound by the immobilized antibody.
Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for COL1 is added to the wells and binds to the combination of capture antibody-COL1 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added.
A colored product TMB is formed in proportion to the amount of COL1 present in the sample.
The reaction is terminated by addition of acid and absorbance is measured.
A standard curve is prepared from seven COL1 standard dilutions and COL1 sample concentration determined

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Materials and Storage.

Store kit components at 4~8 °C unless specified otherwise.
DO NOT USE past kit expiration date. Some vials contain a small amount of reagents.
Spin tubeson pulse setting prior to opening

Components

Component	Size	Cat No.	Srorage of unopened / Reconstituted Materials
Antibody Coated Plate	8×12	tcbe113125-1	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20°C. Reseal along entire edge of zip-seal.
Standard Lyophilized	2	tcbe113125-2	Aliquot and store at ≤ -20 °C in a manual defrost freezer. * Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (100×)	1 ×120ul	tcbe113125-3	May be stored for up to 6 month at 2-8 °C.*
Streptavidin-HRP Concentrated (100×)	1 ×120ul	tcbe113125-4	
Standard/Sample Diluent (R1)	1 ×20mL	tcbe113125-5	
Biotin-Conjugate Antibody Diluent (R2)	1 ×12mL	tcbe113125-6	
Streptavidin-HRP Diluent(R3)	1 ×12mL	tcbe113125-7	
Wash Buffer(30x)	1 ×20mL	tcbe113125-8	
TMB Substrate	1 ×9mL	tcbe113125-9	
Stop Solution	1 ×9mL	tcbe113125-10	
Plate Sealers	4	Ambient Temperature	
Datasheet Operation Manual	1		

Sample Types:

I. Serum Plasma
II.Cell culture supernatant, Body fluids or tissue homogenate.

Specimen Collection and Storage

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4oC before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS (0.02mol/L, pH7.0-7.2) to remove excess blood thoroughly and weighed 300-500mg before homogenization. Minced the tissues to small pieces and homogenized them in 500ul of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm).Remove the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

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Cell lysates - Cells should be lysed according to the following directions.

1) Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
2) Wash cells three times in PBS.As for the collection of the samples, the amount of cells should be no less than 10^8 in 200ul PBS.
3) Cells were resuspended in PBS and subjected to ultrasonication for 3 times.

Alternatively, freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the freeze/thaw cycle for 3 times.
4) Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8 °C to remove cellular debris.
5) Assay immediately or store samples at -20°C or -80°C.

Cell culture supernatants and other body fluids - Centrifuge cell culture media at 1000 × g (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20°C or -80°C.

NOTE:

1) 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.

2) Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.

3) 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.

4) Do not use heat-treated specimens.

Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-250µL
- Plate shaker
- Disposable tips
- Incubator 37°C
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm.

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Preparation of samples.

Taiclone responsible ONLY to the kit but NOT responsible for the samples consumed during the assay.

The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient amount of samples in advance.

2) Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. We suggest pre-experimenting with neat (undiluted) samples,1:2 or 1:4 dilutions. Please avoid diluting your samples more than 1:10 as it would exceed the dilution limit set for this kit. If the expected concentration of the target is beyond the detection range of the kit, please contact technical support.
3) If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4) Owing to the possibility of mismatching between antigen from other resource and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.
5) Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.

Preparation of reagents.

Prepare only the appropriate amount of required reagent on the day of use.

Store all reagents as per instructions stated on the label.

1) Bring all kit components and samples to room temperature (20-25 °C) before use.
2) Samples - Please predict the concentration before assaying. If concentrations are unknown or not within the detection range, a preliminary experiment is recommended to determine the optimal dilution. PBS (pH 7.0-7.2) or 0.9% physiological saline can be used as dilution buffer.
3) Wash Solution - Dilute 10 mL of Wash Solution concentrate (100×) with 990 mL of deionized or distilled 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.
4) Do not dilute the other components which are ready- to-use.

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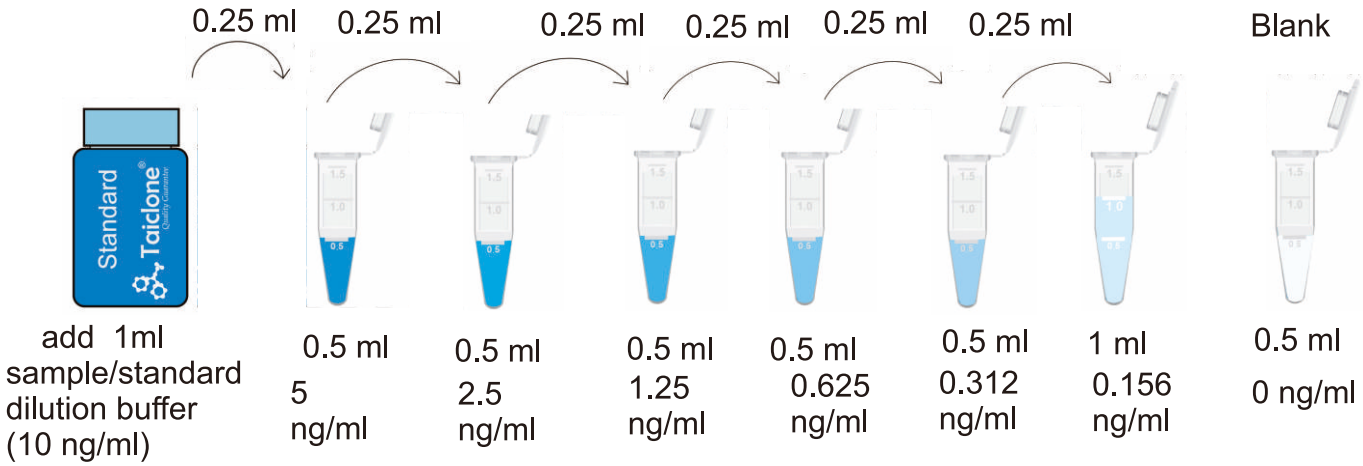
Assay Procedures.

Please read Reagents Preparation before starting assay procedure.
It is recommended that all Standards and Samples be added in duplicate to the microtiter plate.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 100 µL Standard/sample Diluent (R1) in blank well.
3. Add 100 µL different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 1 hour at 37°C.
4. Remove the liquid of each well, don't wash.
5. Prepare the Concentrated Biotin Conjugate Antibody (100X) Working Solution 15 minutes early before use.
6. Add Biotin-Conjugate Antibody Diluent(R2) in blank well and BiotinConjugate antibody Working Solution in other wells (100µL/well), cover with new adhesive strip provided. Incubate for 1 hour at 37°C.
7. Prepare the Streptavidin-HRP Concentrated (100X) Working Solution 15minutes early before use.
8. Add wash buffer 350 µL/well, aspirate each well after holding 60-120 seconds, repeating the process two times for a total of three washes.
9. Add Streptavidin-HRP Diluent(R3) in blank well and add Streptavidin-HRP Working Solution in other wells (100 µL/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
10. Warm-up the Microplate reader.
11. Add wash buffer 350 µL/well, aspirate each well after holding 60-120 seconds, repeating the process four times for a total of five washes.
12. Add TMB Substrate (90µL/well). Incubate for 15-20 minutes at 37°C .Protect from light.
13. Add Stop Solution (50µL/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate.

Readings made directly at 450 nm without correction may be higher and less accurate.



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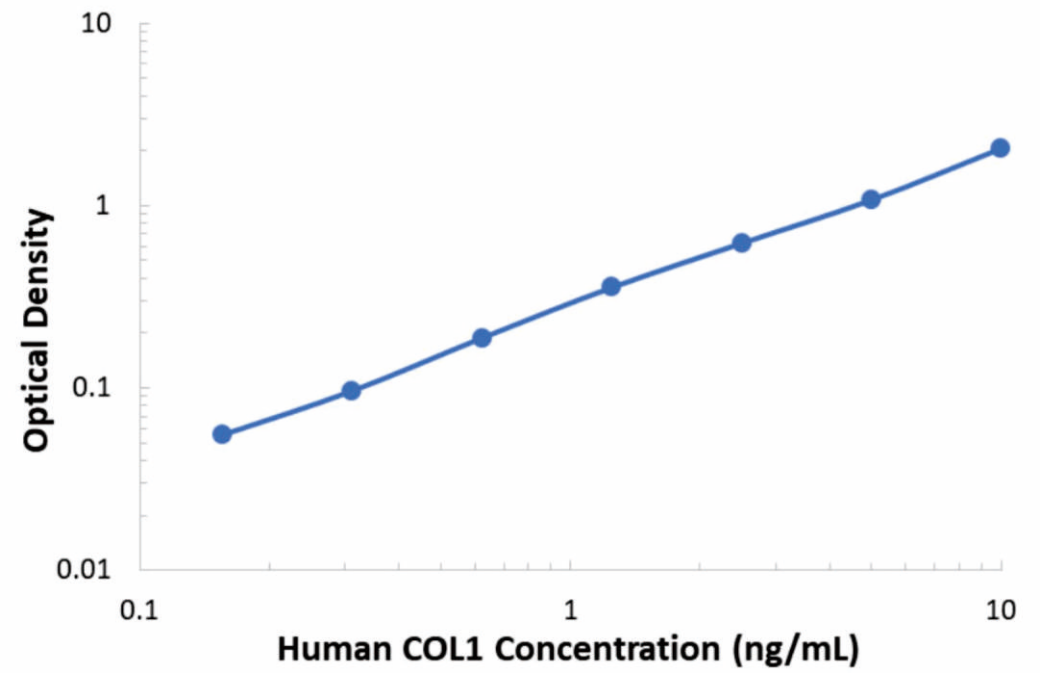
Calculation of result.

1. The standard curve is used to determine the amount of samples.
2. First, 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.
   DO NOT subtract the O.D of standard zero.
3. 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 draw a best fit curve using graph paper or statistical software to generate a four paramater logistic (4-PL) curve-fit or log to-log linear regression curve. An x-axis for the optical density and a y-axis for the concentration is also a choice. The data may be linearized by plotting the log of the concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis.
4. Calculate the concentration of samples corresponding to the mean absorbance from the standard curve.
5. Standard curve for demonstration only.



Note:

1. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.
2. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
3. If specimens generate values higher than the highest standard, dilute the specimens and repeat the assay.

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Recovery :

Spike 3 different concentration of human COL1 into healthy serum and plasma, calculate the recovery.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	85-99	90
EDTA plasma(n=5)	80-101	95
heparin plasma(n=5)	78-91	86

Precision : Intraplate

3 samples with low, middle and high level COL1 were tested 20 times on one plate, respectively.
Intra-Assay: CV<10%

Precision : Interplate

3 samples with low, middle and high level COL1 were tested on 3 different plates, 8 replicates in each plate.
Inter-Assay: CV<12%

Linearity:

Spike high concentration of human COL1 into 4 healthy serum and plasma, dilute in the range of standard curve kinetics and evaluate the linearity

Sample	1:2	1:4	1:8	1:16
serum(n=5)	90-104%	80-97%	84-102%	93-106%
EDTA plasma(n=5)	84-101%	88-102%	85-92%	90-99%
heparin plasma(n=5)	83-93%	82-96%	91-103%	81-98%

Safety Precautions

• The test protocol must be followed strictly.
• All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
• The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine (TMB). Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
• Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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• Only trained laboratory personnel should execute this test.

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Quality Control.

1. It is recommended that all standards, controls and samples be run in duplicate.
   Standards and samples must be assayed at the same time.
2. The coefficient of determination of the standard curve should be ≥ 0.95 and the highest O.D.
   should be more than 1.0.
3. Cover or cap all kit components and store at 2-8° C when not in use.
4. Microtiter plates should be allowed to come to room temperature before opening the foil bags.
   Once the desired number of strips has been removed, immediately reseal the bag with
   desiccants and store at 2-8°C to maintain plate integrity.
5. Samples should be collected in pyrogen/endotoxin-free tubes.
6. Samples should be frozen if not analyzed shortly after collection.
   Avoid multiple freeze-thaw cycles of frozen samples.
   Thaw completely and mix well prior to analysis.
7. When possible, avoid use of badly hemolyzed or lipemic serum.
   If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well.
   This ensures equal incubation times for all wells.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbance immediately after adding the stop solution.
12. 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.
13. Because TMB is light sensitive, avoid prolonged exposure to light.
    Also avoid contact between TMB and metal, otherwise color may develop.

Taiclone Biotech Corp.

Tel :+886 2 2735 9682
Fax:+886 2 2735 9807
Email: order@taiclone.com
Website:www.taiclone.com