Chondrex, Inc.

Human Type I Collagen Detection Kit

Catalog # 6021

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

Type I collagen is a fibrillar collagen consisting of two identical α1(I) chains and one α2(I) chain. It is the most abundant collagen type and is found in most connective tissues such as skin, bone, tendon, ligament, and heart. Many tissues contain heterotypic fibrils meaning that two or more distinct collagen types coexist. For example, human skin contains heterotypic fibrils of type I collagen (90%) and type III collagen (10%).

This new Human Type I Collagen Detection Kit (catalog # 6021) was developed with a new capture antibody and detection antibody to improve specificity against human type I collagen compared with our previous kit (catalog # 6008).

Antibody Specificity		
Collagen Type	**Capture Antibody**	**Detection Antibody (Biotinylated)**
Human I	100%	100%
Denatured Human I	1%	6%
Human II	0%	33%
Human III	3%	100%
Bovine I	0%	0%
Mouse I	0%	1%
Chick I	0%	1%
Porcine I	0%	1%
Rat I	0%	1%

ANTIBODY SPECIFICITY

A pair of monoclonal antibodies to native human type I collagen is used as the capture and detection antibodies in this kit. Both of the antibodies are highly specific to the native conformation of type I collagen, therefore this kit cannot detect denatured human type I collagen in samples. In addition, since the capture antibody is highly specific to human type I collagen, this kit is ideally used to measure human type I collagen in samples mixed with other species of type I collagen (see table above).

KIT COMPONENTS

Item	Catalog #	Quantity	Amount	Storage
Human Type I Collagen Standard	60081	1 vial	100 µl, 100 µg/ml	-20°C
Capture Antibody	60212	1 vial	100 µl, 1 mg/ml	-20°C
Detection Antibody	60213	1 vial	Lyophilized	-20°C
Solution A - Capture Antibody Dilution Buffer	9052	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer	9053	1 bottle	50 ml	-20°C
Solution C - Detection Antibody Dilution Buffer	9054	1 bottle	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer	9055	1 bottle	20 ml	-20°C
Streptavidin Peroxidase	9029	2 vials	50 µl	-20°C
OPD	90021	2 vials	Lyophilized	-20°C
Chromagen Dilution Buffer	90022	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	9016	1 bottle	10 ml	-20°C
Wash Buffer, 20X	9005	1 bottle	50 ml	-20°C
ELISA Plate	9026	1 each	96-well (8-well strips x 12)	-20°C

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NOTES ON PROCESSING SAMPLES

Solubilization of Collagen

For determining collagen contents in cultured cell layers and tissues by ELISA, solubilization of collagen is required. To solubilize collagen from sample specimens, a limited digestion of tissue specimens with pepsin is recommended, although other neutral proteinases, such as pronase and papain are capable of solubilizing collagen. These proteinases only digest telopeptides located on both N- and C-terminal of the collagen molecule, but are not capable of digesting the helical conformation region of the collagen molecule and intra- and inter-molecular cross-linkages, thus leaving the collagen molecule intact (atelocollagen).

The solubilization of collagen from tissues by a limited proteinase digestion (generally collagen/proteinase ratio is 100:1) depends on the types of tissues and the contents of intra- and inter-molecular cross-linkages. For example, bone and Achilles tendon are resistant to pepsin digestion, and only 10-20% of collagen tissue will be solubilized. On the other hand, young calf skin collagen will be completely solubilized by pepsin digestion within 24-48 hours, but it takes 7-9 days to solubilize adult calfskin.

Therefore, proteinase resistant insoluble collagen might be solubilized by alkaline treatment. Suspend insoluble collagen in cold 3% NaOH solution containing 1.9% monomethylamine, and incubate at 4°C for 1-2 weeks. After treatment of collagen with alkaline, dialyze against 0.05M acetic acid or neutral buffer such as 0.1M Tris-0.3M NaCl, pH 7.5.

Therefore, the optimum solubilization condition for individual samples should be determined before processing samples. Collagen can be analyzed by 6% SDS-gel under non-reducing condition, using authentic type I collagen as a standard. If samples contain bands larger than the γ-chain (MW = 300 Kd), the samples must be further digested by pepsin or elastase. On the other hand, if smaller bands or smear bands are observed under the α-chain (MW = 100 Kd), the samples might be over-digested. Therefore, it is critical to understand the biological and physico-chemical properties of individual collagen samples.

"Tips for Collagen Solubulization" can be obtained from Chondrex customer service.

NOTES BEFORE USING ASSAY

Note 1: It is recommended that the standard and samples be run in duplicate.

Note 2: Ensure all reagents are at room temperature before proceeding.

Note 3: Partially used reagents may be kept at –20°C. If several assays are expected, reconstitute the Detection Antibody with 50 µl of distilled water, then use the solution as needed. The remaining antibody solution can be stored at -20°C. For example, mix 5 µl of reconstituted solution in 1 ml of Solution C. Similarly, the Capture Antibody can be used partially and stored at -20°C. For example, mix 10 µl of Capture Antibody with 1 ml of Solution A and use for plate coating.

Note 4: Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, it is necessary to warm the wash buffer by placing the bottle in warm water until crystals have dissolved completely.

Note 5: Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.

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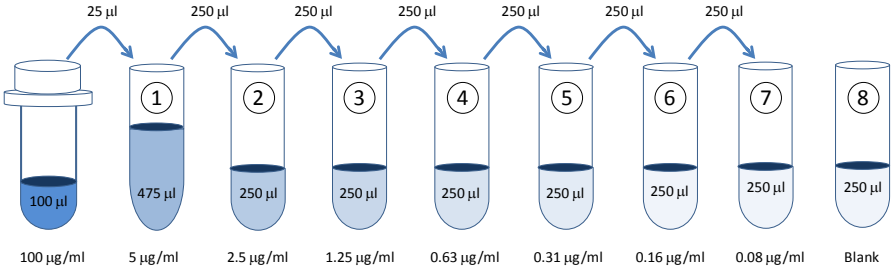
ASSAY PROCEDURE

1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Capture Antibody Dilution Buffer (Solution A). Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight.

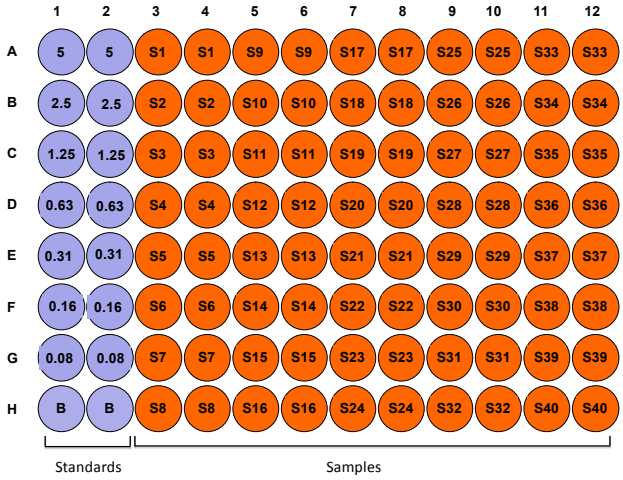
2. **Dilute Wash Buffer**: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

3. **Prepare Standard Dilutions**: The standard range is 0.08-5 µg/ml. Prepare serial dilutions of the standard by mixing 25 µl of 100 µg/ml standard with 475 µl of Sample/Standard Dilution Buffer (Solution B) - 5 µg/ml. Then mix 250 µl of the 5 µg/ml standard with 250 µl of Solution B - 2.5 µg/ml. Repeat this procedure to make five more serial dilutions of standard - 1.25, 0.63, 0.32, 0.16, and 0.08 µg/ml solutions. The 100 µg/ml standard stock may be stored at -20°C for use in a second assay. We recommend making fresh serial dilutions for each assay.



4. **Prepare Sample Dilutions**: Dilute solubilized samples 1:1-1:1000 with Solution B depending on samples. Samples must be diluted at least 1:1 to maintain optimal assay conditions. Do not use undiluted samples.

5. **Add Standards and Samples**: Mix samples and standard tubes well. Add 100 µl of Solution B (blank), standards and samples to appropriate wells. Incubate at room temperature for 2 hours.



6. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

7. **Add Detection Antibody**: Dissolve one vial of Detection Antibody in 10 ml of Detection Antibody Dilution Buffer (Solution C). Add 100 µl of detection antibody solution to each well and incubate at room temperature for 2 hours.

8. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

9. **Add Streptavidin Peroxidase**: Dilute one vial of Streptavidin Peroxidase in 10 ml of Streptavidin Peroxidase Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

10. **Wash**: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **OPD**: Dissolve one vial of OPD in 10 ml of Chromagen Dilution Buffer just prior to use. Add 100 µl of OPD solution to each well immediately after washing the plate. Incubate for 30 minutes at room temperature.

12. **Stop**: Add 50 µl of 2N sulfuric acid (Stop Solution) to each well.

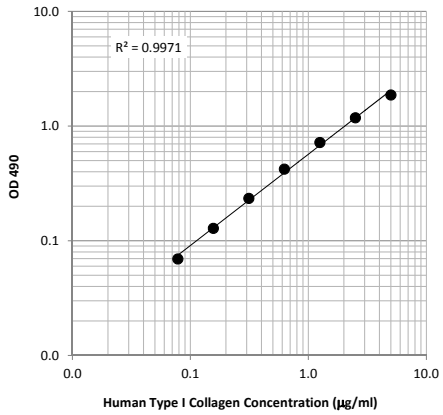
13. **Read Plate**: Read the OD values at 490 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

CALCULATION OF RESULTS

1. Average the duplicate OD values for the blank, standards, and test samples.

2. Subtract the "blank" (B) values from the averaged OD values in step 1.

3. Plot the OD values of standards against the concentration of collagen (µg/ml). Using a log/log plot will linearize the data. The following figure shows a representative experiment where the standard range is from 0.08 to 5 µg/ml.



4. The µg/ml of human type I collagen in test samples can be calculated using regression analysis.

Reproducibility of Data Assayed by Human Type I Collagen Detection Kit

Test At	2.5 µg/ml	0.63 µg/ml	0.16 µg/ml
Inter-Assay CV (%)	4.3	2.0	6.6
Intra-Assay CV (%)	3.2	3.9	1.4
Spiking Test (%)*	99.6	98.6	105.6

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