Chondrex, Inc.

Rat Type I Collagen Detection Kit

Catalog # 6013

For Research Use Only

INTRODUCTION

Type I collagen is one of several interstitial fibrillar collagens consisting of two identical 1(I) chains and one 2(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, most connective tissues (with the exception of bone) contain heterotypic fibrils of type I and III collagen, although type III collagen is minor. The Rat Type I Collagen Detection Kit is designed to quantify the amount of type I collagen in cultured rat cells and tissue specimens by ELISA. All necessary reagents to measure 39 samples, in duplicate, are included in the kit. The sensitivity of this assay is approximately 0.08-5 μg/ml.

Antibody Specificity		
Collagen Type	**Capture Antibody**	**Detection Antibody (Biotinylated)**
Rat I	100%	100%
Rat Denatured I	2%	3%
Rat II	0%	0%
Rat III	Not Determined	Not Determined
Bovine I	0%	100%
Chick I	50%	0%
Human I	0%	0%
Mouse I	0%	0%
Porcine I	0%	75%

ANTIBODY SPECIFICITY

A pair of mouse IgG monoclonal antibodies specific to native rat type I collagen are used as the capture and detection antibodies in the Rat Type I Collagen Detection Kit. Both of these clones are highly specific to the native conformation of type I collagen and poorly cross-react with denatured type I collagen. This kit can be used to determine rat type I collagen in samples including other species of type I collagen and type II collagen because the capture antibody is highly specific to rat type I collagen and does not cross-react with other species of type I collagen (except chick) and type II collagen (see table above).

KIT COMPONENTS

Item	Quantity	Amount	Storage
Rat Type I Collagen Standard	1 vial	100 μl, 100 μg/ml	-20°C
Capture Antibody	1 vial	100 μl, 1 mg/ml	-20°C
Detection Antibody	1 vial	50 μl, lyophilized	-20°C
Solution A - Capture Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution B - Sample/Standard Dilution Buffer	1 bottle	50 ml	-20°C
Solution C - Detection Antibody Dilution Buffer	1 bottle	10 ml	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer	1 bottle	20 ml	-20°C
Streptavidin Peroxidase	2 vials	50 μl	-20°C
OPD	2 vials	Lyophilized	-20°C
Chromagen Dilution Buffer	1 bottle	20 ml	-20°C
Stop Solution - 2N Sulfuric Acid	1 bottle	10 ml	-20°C
Wash Buffer, 20X	1 bottle	50 ml	-20°C
ELISA Plate	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. For solubilizing 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.

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.

Proteinase resistant insoluble collagen might be solubilized by alkaline treatment. Suspend insoluble collagen in cold 0.1N NaOH solution containing 10% Na_2SO_4 and 0.1M Amine such as Tris, and incubate at 4°C for 1-2 weeks. After treatment of collagen with alkaline, neutralize the pH to 5.0 with HCl, and then dilute it with 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 on solubilization of collagen 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: Partially used reagents may be kept at –20°C.

Note 3: 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 4: 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 recommended 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. Then 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 tissue samples 1:1-1:1000 with Solution B depending on the estimated collagen content in the samples. Cell samples can be used without further dilution. However, if it is necessary, dilute cell samples 1:1-1:2 with Solution B.

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.

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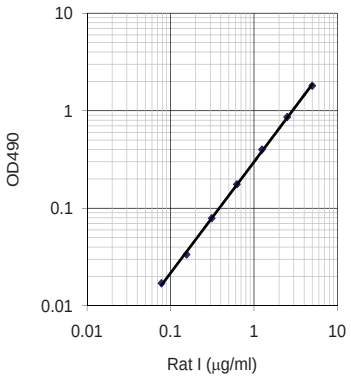
CALCULATION OF RESULTS

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. Figure 3 shows a representative experiment where the standard range is from 0.08 to 5 μg/ml.
4. The μg/ml of rat type I collagen in test samples can be calculated using regression analysis.

Figure 3 - A typical standard curve



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