Type I Collagen Detection ELISA Kits

Catalog # 6012, 6013, 6014, 6015, 6016, 6019, and 6021

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kits to quantify Type I Collagen
	6012: Mouse Type I Collagen Detection ELISA Kit
	6013: Rat Type I Collagen Detection ELISA Kit
	6014: Bovine Type I Collagen Detection ELISA Kit
	6015: Porcine Type I Collagen Detection ELISA Kit
	6016: Rabbit Type I Collagen Detection ELISA Kit
	6019: Canine Type I Collagen Detection ELISA Kit
	6021: Human Type I Collagen Detection ELISA Kit
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	5.5 hours
STANDARD RANGE:	6012/6013/6015/6021: 5 µg/ml to 0.08 µg/ml
	6014/6016/6019: 10 µg/ml to 0.16 µg/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Solubilized collagen and cell culture media
RECOMMENDED SAMPLE DILUTIONS:	1:1 - 1:1000
CHROMOGEN:	OPD (read at 490 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	6021 (only): Intra-assay (1.4-3.9%)/Inter-assay (2-6.6%)/Spiking Test (99-106%)
NOTES:	These kits are intended to detect native type I collagen and will only weakly detect denatured collagen

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16928 Woodinville-Redmond Rd NE Suite B-101
Woodinville, WA 98072
Phone: 425.702.6365 or 888.246.6373
Fax: 425.882.3094
www.chondrex.com
support@chondrex.com

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INTRODUCTION

Type I collagen is one of several interstitial fibrillar collagens 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, most connective tissues (except for bone) contain heterotypic fibrils of type I and III collagen, although the type III collagen content is minor compared to Type I collagen. Chondrex, Inc. provides a variety of species-specific Type I Collagen Detection ELISA Kits designed to quantify the amount of species-specific type I collagen in cultured cells and tissue specimens.

ANTIBODY SPECIFICITY

Monoclonal antibodies are used as the capture and detection antibodies in these Type I Collagen Detection ELISA Kits. All clones are highly specific to the native conformation of their respective type I collagens and poorly cross-react with denatured type I collagen. Chondrex, Inc. recommends carefully selecting kits to determine species-specific type I collagen in samples containing other species of type I collagen or type II collagen based on the specificity of the capture antibodies in the table below. Some capture antibodies are highly specific to their respective type I collagens and do not cross-react with other species of type I or type II collagen.

Collagen Type	Mouse		Rat		Bovine		Porcine		Rabbit		Canine		Human	
	CA	DA	CA	DA	CA	DA	CA	DA	CA	DA	CA	DA	CA	DA
Mouse I	100%	100%	0%	0%	31%	0%	31%	0%	31%	0%	31%	0%	0%	1%
Mouse Denatured I	5%	3%	-	-	-	-	-	-	-	-	-	-	-	-
Mouse II	0%	0%	-	-	-	-	-	-	-	-	-	-	-	-
Mouse III	ND	ND	-	-	-	-	-	-	-	-	-	-	-	-
Rat I	0%	5%	100%	100%	-	-	-	-	-	-	0%	100%	0%	1%
Rat Denatured I	-	-	2%	3%	-	-	-	-	-	-	-	-	-	-
Rat II	-	-	0%	0%	-	-	-	-	-	-	-	-	-	-
Rat III	-	-	ND	ND	-	-	-	-	-	-	-	-	-	-
Bovine I	0%	24%	0%	100%	100%	100%	100%	100%	100%	100%	100%	100%	0%	0%
Bovine Denatured I	-	-	-	-	3%	3%	-	-	-	-	-	-	-	-
Bovine II	-	-	-	-	0%	0%	-	-	-	-	-	-	-	-
Bovine III	-	-	-	-	ND	ND	-	-	-	-	-	-	-	-
Porcine I	0%	56%	0%	75%	-	-	100%	75%	100%	75%	100%	75%	0%	1%
Porcine Denatured I	-	-	-	-	-	-	3%	4%	-	-	-	-	-	-
Porcine II	-	-	-	-	-	-	0%	0%	-	-	-	-	-	-
Porcine III	-	-	-	-	-	-	ND	ND	-	-	-	-	-	-
Rabbit I	-	-	-	-	-	-	-	-	100%	70%	-	-	-	-
Rabbit Denatured I	-	-	-	-	-	-	-	-	2%	1%	-	-	-	-
Rabbit II	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rabbit III	-	-	-	-	-	-	-	-	ND	ND	-	-	-	-
Canine I	-	-	-	-	-	-	-	-	-	-	100%	100%	-	-
Canine Denatured I	-	-	-	-	-	-	-	-	-	-	5%	5%	-	-
Canine II	-	-	-	-	-	-	-	-	-	-	0%	0%	-	-
Canine III	-	-	-	-	-	-	-	-	-	-	ND	ND	-	-
Human I	0%	0%	0%	0%	100%	0%	100%	0%	100%	0%	100%	0%	100%	100%
Human Denatured I	-	-	-	-	-	-	-	-	-	-	-	-	1%	6%
Human II	-	-	-	-	-	-	-	-	-	-	-	-	0%	33%
Human III	-	-	-	-	-	-	-	-	-	-	-	-	3%	100%
Chick I	0%	0%	50%	0%	0%	0%	0%	0%	0%	0%	0%	0%	0%	1%
Chick Denatured I	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chick II	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chick III	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*CA: Capture Antibody
**DA: Detection Antibody (Biotinylated)
***ND: No Data

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KIT COMPONENTS

Item		Quantity	Amount	Storage
Standard	Mouse (60121) Rat (60131) Bovine (60141) Porcine (60151) Rabbit (60161) Canine (60191) Human (60081)	1 vial	100 µl (Mouse, Rat, Porcine, Human) 150 µl (Bovine, Rabbit, Canine) 100 µg/ml	-20°C
Capture Antibody	Mouse (60122) Rat (60132) Bovine (60142) Porcine (60152) Rabbit (60162) Canine (60192) Human (60212)	1 vial	100 µl, 1 mg/ml	-20°C
Detection Antibody	Mouse (60123) Rat (60133) Bovine (60143) Porcine (60153) Rabbit (60163) Canine (60193) Human (60213)	1 vial	Lyophilized	-20°C
Solution A - Coating 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
Chromogen 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		1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

Add 100 µl of capture antibody solution into wells

Incubate at 4 degrees C overnight.
Wash plate.

Add 100 µl of diluted standards and samples into wells

Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted detection antibody solution into wells

Incubate at room temperature for 2 hours.
Wash plate.

Add 100 µl of diluted streptavidin peroxidase solution into wells

Incubate at room temperature for 1 hour
Wash plate.

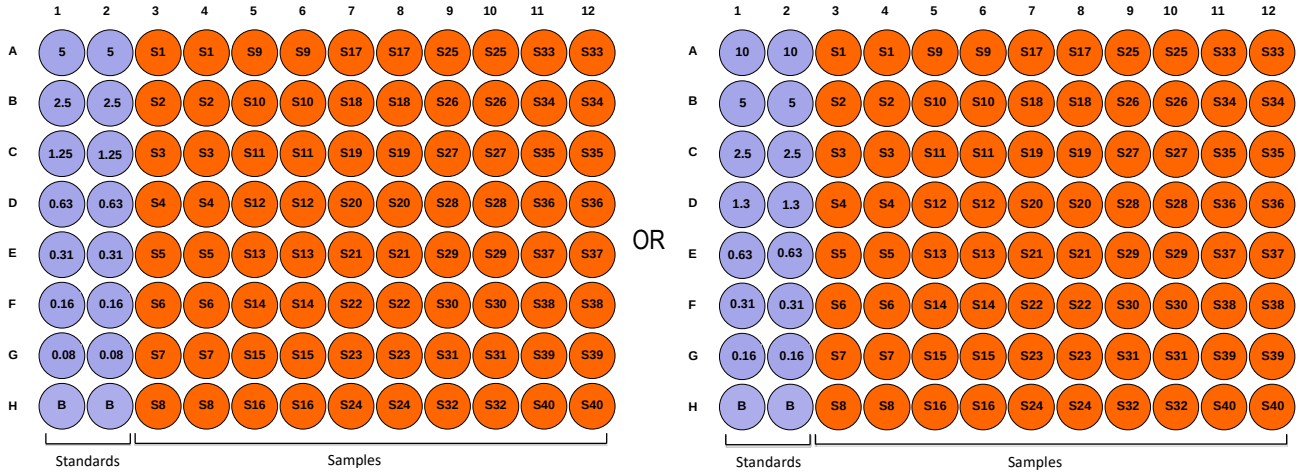
Add 100 µl of OPD solution into wells

Incubate at room temperature for 30 minutes.

Add 50 µl of Stop Solution into wells

Read plates at 490 nm/630 nm

PLATE MAPPING



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SOLUBILIZING COLLAGEN

For determining collagen content in cultured cell layers and tissues by ELISA, solubilizing collagen is required. For tissue specimens, a limited digestion with pepsin is highly recommended for native collagen preparation as other neutral proteinases, such as pronase and papain, digest collagen into small peptides. Pepsin only digests telopeptides located on both the N- and C-terminals of the collagen molecule and is not capable of digesting the helical conformation region of the collagen molecule itself. Please inquire with Chondrex, Inc. customer service for "Tips on Solubilization of Collagen".

Solubilizing collagen from tissues by limited pepsin digestion (generally collagen to pepsin ratio is 100:1) depends on the types of tissues and the contents of the intra- and inter-molecular cross-linkages. For example, bone and Achilles tendon are resistant to pepsin digestion and only 10-20% of the collagen tissue will be solubilized. Young calf skin collagen will be completely solubilized by pepsin digestion within 24-48 hours, while it takes 7-9 days to solubilize adult skin. Pepsin 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 conditions, 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 which will digest the intra- and inter-collagen cross-linkages. 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.

NOTES BEFORE USING ASSAY

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

NOTE 2: Warm up all buffers to room temperature before use.

NOTE 3: Crystals may form in Wash Buffer, 20X when stored at cold temperatures. If crystals have formed, warm the wash buffer by placing the bottle in warm water until crystals are completely dissolved.

NOTE 4: Measure exact volume of buffers using a serological pipet, as extra buffer is provided.

NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

NOTE 6: For partial reagent use, please see the assay protocol's corresponding step for the appropriate dilution ratio. For example, if the protocol dilutes 50 µl of a stock solution in 10 ml of buffer for 12 strips, then for 6 strips, dilute 25 µl of the stock solution in 5 ml of buffer. Partially used stock reagents may be kept in their original vials and stored at -20ºC for use in a future assay.

NOTE 7: This kit contains animal components from non-infectious animals and should be treated as potential biohazards in use and for disposal.

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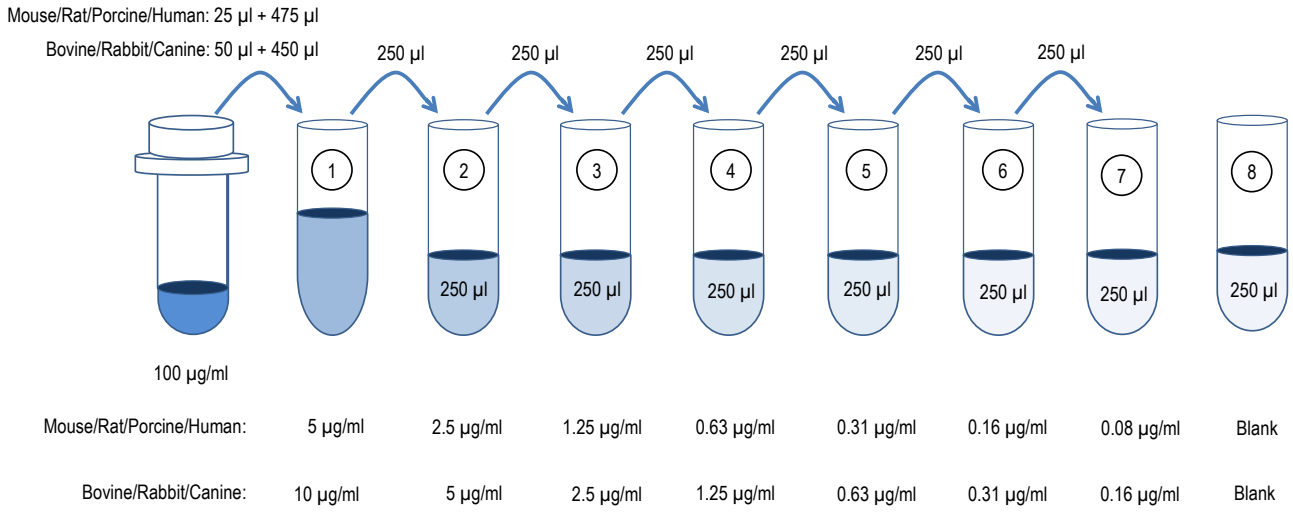
ASSAY PROCEDURE

1. **Add Capture Antibody**: Dilute one vial of Capture Antibody with 10 ml of Coating Buffer (Solution A). Alternatively, dilute according to assay needs. Add 100 µl of capture antibody solution to each well and incubate at 4°C overnight. Any remaining Capture Antibody Stock Solution can be stored at -20°C for future use.

Strip #	Capture Antibody (µl)	Solution A (ml)
2	17	1.7
4	33	3.3
6	50	5.0
8	66	6.6
10	82	8.2
12	100	10.0

2. **Prepare Standard Dilutions**: Please see the figure below for each assay's recommended standard range. Dilute the standard stock accordingly to get the first stock solution. Then serially dilute it with Solution B. For example, mix 250 µl of the first stock solution with an equal volume of Solution B to make the second stock solution, and then repeat it five more times. The original standard stock solution may be stored at -20ºC for use in a future assay. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



3. **Prepare Sample Dilutions**: Dilute tissue samples 1:1-1:1000 with Solution B depending on the estimated collagen content in the samples. The samples must be diluted with Solution B to maintain optimal assay conditions.

   NOTE: Cell samples and culture media can be assayed at a 1:1 dilution with Solution B.

4. **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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out*.

5. **Add Standards and Samples**: Add 100 µl of standards, Solution B (blank), and samples to appropriate wells in duplicate. 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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

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7. **Add Detection Antibody**: Dissolve one vial of Detection Antibody in 10 ml Detection Antibody Dilution Buffer (Solution C). Alternatively, dissolve one vial of Detection Antibody in 50 µl of Solution C and dilute accordingly. Add 100 µl of detection antibody solution to each well and incubate at room temperature for 2 hours.

Strip #	Detection Antibody (µl)	Solution C (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

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 blotting 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 Dilution Buffer (Solution D). Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 1 hour.

Strip #	Streptavidin Peroxidase (µl)	Solution D (ml)
2	8	1.7
4	17	3.3
6	25	5.0
8	33	6.6
10	42	8.2
12	50	10.0

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 blotting on a paper towel to remove excess liquid. *Do not allow the plate to dry out.*

11. **Add OPD**: Dissolve one vial of OPD with 10 ml of Chromogen Dilution Buffer just prior to use. Add 100 µl of OPD solution to each well immediately after washing the plate and 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.

CALCULATING RESULTS

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

2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

3. Plot the OD values of standards against the concentration of standards (µg/ml). Using a log/log plot will linearize the data.

4. The µg/ml of collagen in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the collagen concentration (µg/ml) in the original sample specimens.

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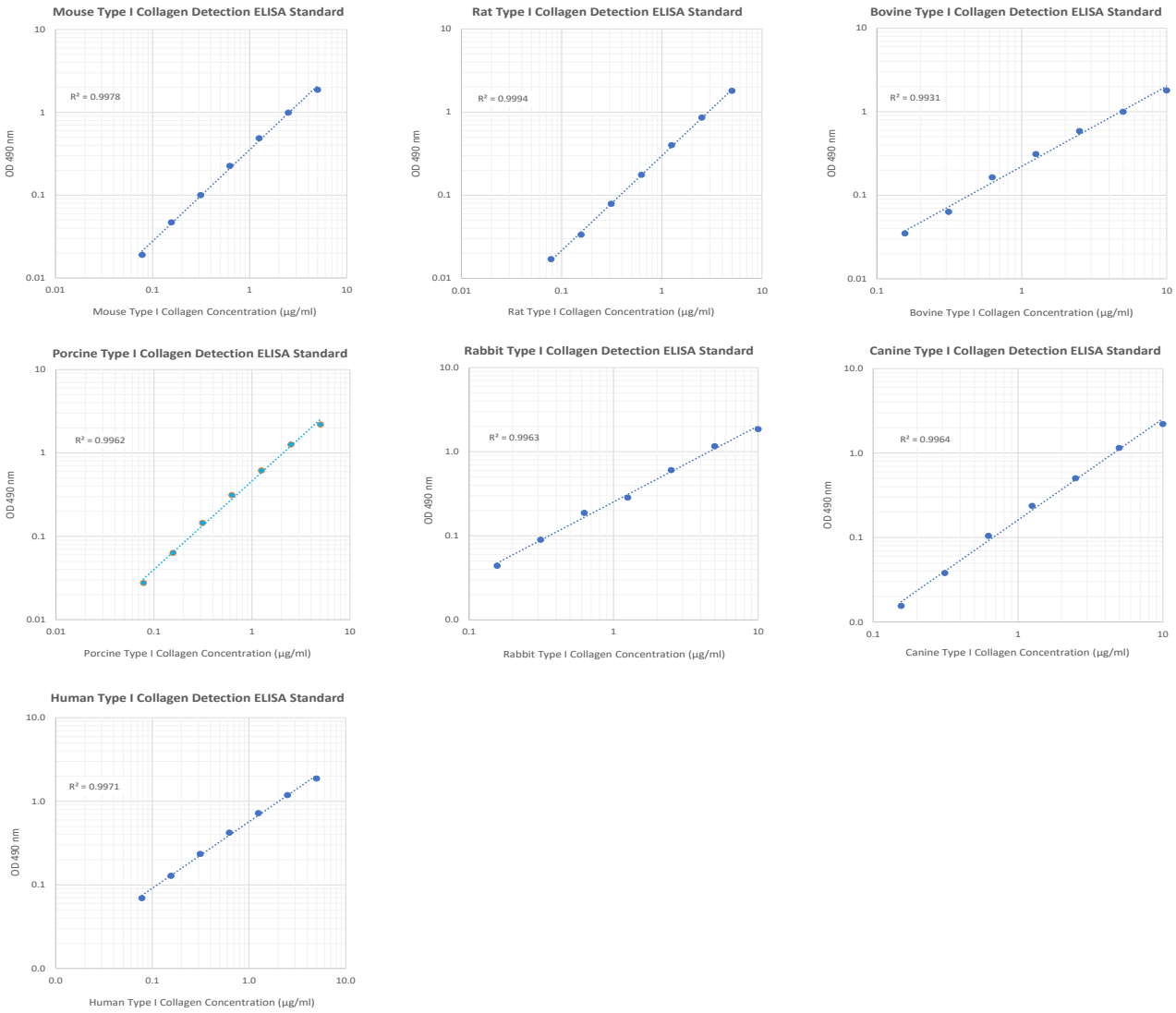
Figure 1 - Typical Standard Curves for the Type I Collagen Detection ELISA Kits



VALIDATION DATA

Table 1 - Reproducibility Data for the Human Type I Collagen Detection ELISA Kit

Test	0.16 µg/ml	0.63 µg/ml	2.5 µg/ml
Intra-Assay CV (%)	1.4	3.9	3.2
Inter-Assay CV (%)	6.6	2.0	4.3
Spike Test* (%)	106%	99%	100%

* Known amounts of Human Type I collagen were added to samples and then diluted with Solution B for assaying Human Type I collagen.

TROUBLESHOOTING

For frequently asked questions about assays and ELISAs, please see Chondrex, Inc.'s ELISA FAQ for more information.

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