Mouse Aggrecan Detection ELISA Kit

Catalog # 6058

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	ELISA kit to quantify mouse aggrecan
FORMAT:	Precoated 96-well ELISA Plate with removeable strips
ASSAY TYPE:	Sandwich ELISA
ASSAY TIME:	4 hours
STANDARD RANGE:	1.6 – 100 ng/ml
NUMBER OF SAMPLES:	Up to 40 (duplicate) samples/plate
SAMPLE TYPES:	Serum, Plasma, and biological fluids (pre-treatment acceptable)
RECOMMENDED SAMPLE DILUTIONS:	1:1 (at least)
CHROMOGEN:	TMB (read at 450 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (7.0-8.3%)/Inter-Assay (5.5-8.8%)/Spiking Test (92-100%)
NOTES:	N/A

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INTRODUCTION

Aggrecan, also known as cartilage-specific proteoglycan core protein or chondroitin sulfate proteoglycan 1, is a large proteoglycan vital for cartilage structure and function [1]. The protein core of aggrecan is approximately 250 kDa and is predominantly expressed in cartilage, particularly in articular cartilage, although it is also present in other connective tissues. Aggrecan exhibits a bottlebrush structure, in which chondroitin sulfate and keratan sulfate chains are attached to an extended protein core. This structure enables the formation of highly hydrated, gel-like assemblies that are critical for the load-bearing capacity and resilience of cartilage [2]. Aggrecan contains three interglobular domains, G1, G2, and G3, and interacts with hyaluronic acid and link proteins. [3].

These aggregates impart osmotic properties to cartilage, allowing it to resist compressive forces during joint movement. Consequently, aggrecan plays a central role in maintaining normal cartilage function and joint health. Aggrecan fragments from articular cartilage are released into the synovial fluid at all stages of osteoarthritis due to cleavage by aggrecanases and matrix metalloproteinase activity. The synthesis and degradation of aggrecan is being investigated with respect to their roles in cartilage deterioration during joint injury, disease, and aging [4-7].

Chondrex, Inc. provides a quantitative Mouse Aggrecan Detection ELISA Kit (Cat # 6058) using two anti-G1 domain monoclonal antibodies for measuring aggrecan levels in biological fluids and serum samples. Chondrex, Inc. also offers Hyaluronan Detection Kits (Cat # 6048 and # 6049). Together, these products can be used to study aggrecan–hyaluronic acid interactions within the extracellular matrix in experimental systems.

NOTE: this kit can also detect human aggrecan in samples because the monoclonal antibodies can cross-react with the human aggrecan G1 domain.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Mouse Aggrecan G1 Domain Standard (60581)	1 vial	100 ng, lyophilized	-20°C
Anti-Aggrecan G1 Detection Antibody (60583)	1 vial	50 µl	-20°C
Solution C - Sample/Standard/Detection Antibody Dilution Buffer (30106)	1 bottle	50 ml	-20°C
Streptavidin Peroxidase (9029)	2 vials	50 µl	-20°C
Solution D - Streptavidin Peroxidase Dilution Buffer (9055)	1 bottle	20 ml	-20°C
TMB Solution (90023)	2 vials	0.2 ml	-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
Anti-Mouse Aggrecan G1 Antibody Coated ELISA Plate (Blue)	1 each	96-well (8-well strips x 12)	-20°C

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ASSAY OUTLINE

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

↓ Incubate at room temperature for 1 hour.
Wash plate.

Add 100 µl of diluted Streptavidin Peroxidase

↓ Incubate at room temperature for 30 minutes.
Wash plate.

Add 100 µl of TMB solution

↓ Incubate at room temperature for 25 minutes.

Add 50 µl of Stop Solution

↓

Read plates at 450 nm/630 nm

PLATE MAPPING

	1	2	3	4	5	6	7	8	9	10	11	12
A	100	100	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33
B	50	50	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34
C	25	25	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35
D	12.5	12.5	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36
E	6.3	6.3	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
F	3.1	3.1	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
G	1.6	1.6	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
H	B	B	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40

Standards | Samples

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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.

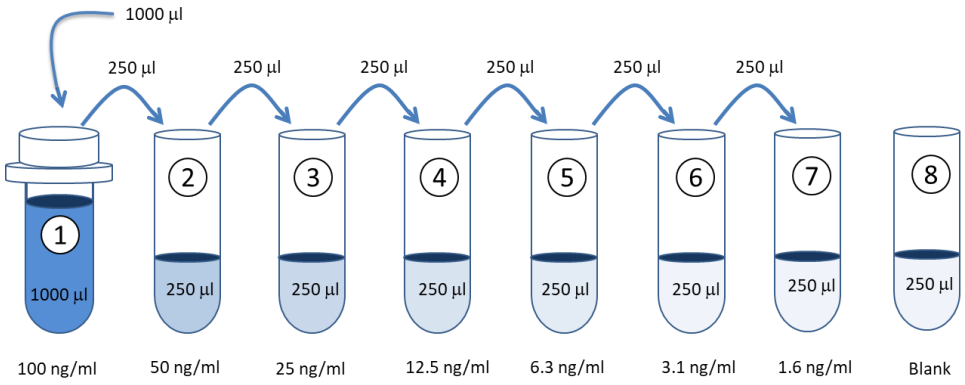
NOTE 5: Cover the plate with plastic wrap or a plate sealer after each step to prevent evaporation from the outside wells of the plate.

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.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: The recommended standard range is 1.6 - 100 ng/ml. Dissolve one vial of Standard (100 ng/vial) in 1 ml of Sample/Standard Dilution Buffer (Solution C) and keep it as standard stock. Then serially dilute it with Solution C. For example, mix 250 µl of the 100 ng/ml solution with an equal volume of Solution C to make a 50 ng/ml solution, and then repeat it five more times for 25, 12.5, 6.3, 3.1, and 1.6 ng/ml standard solutions.



2. **Prepare Sample Dilutions**: Dilute samples at least 1:1 with Solution C depending on the estimated mouse aggrecan level in the samples. Two or three different sample dilutions are recommended if the mouse aggrecan levels in the samples are unknown.

3. **Add Standards and Samples**: Add 100 µl of standards, Solution C (blank), and samples to wells in duplicate. Incubate at room temperature for 2 hours.

4. **Wash**: 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 Detection Antibody**: Dilute one vial of Detection Antibody in 10 ml Solution C. Add 100 µl of Detection antibody solution to each well and incubate at room temperature for 1 hour.

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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

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.*

7. **Add Streptavidin Peroxidase Solution**: Prepare streptavidin peroxidase solution with Solution D as shown in the following table. Add 100 µl of streptavidin peroxidase solution to each well and incubate at room temperature for 30 minutes.

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

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 TMB Solution**: Use new tubes when preparing TMB solution. Just prior to use, prepare TMB solution with Chromogen Dilution Buffer as shown in the following table. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature

Strip #	TMB (µl)	Chromogen Dilution Buffer (ml)
2	34	1.7
4	66	3.3
6	100	5.0
8	132	6.6
10	164	8.2
12	200	10.0

10. **Stop**: Stop the reaction with 50 µl of 2N Sulfuric Acid (Stop Solution) to each well.

11. **Read Plate**: Read the OD values at 450 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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CALCULATING RESULTS

1. Average the duplicate OD values for the standards, blanks (B), 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 the standards against the ng/ml of mouse aggrecan standard. Using a log/log plot will linearize the data. Figure 1 shows an example of a standard curve where the standard range is 1.6 to 100 ng/ml.

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

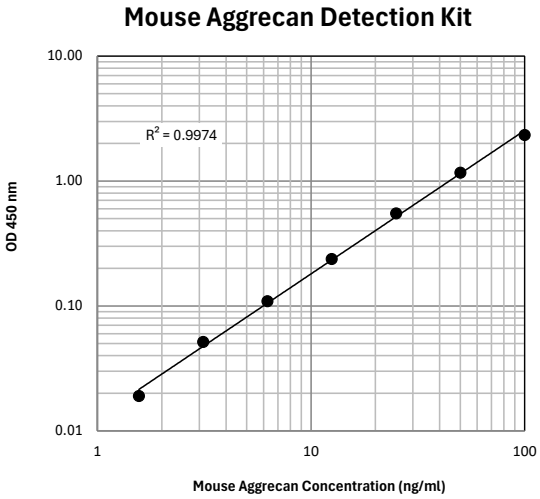

Figure 1 - A Typical Standard Curve for the Mouse Aggrecan Detection ELISA Kit

VALIDATION DATA

Table 1 - Reproducibility Data for the Mouse Aggrecan Detection ELISA Kit

Test	3.1 ng/ml	12.5 ng/ml	50 ng/ml
Intra-Assay CV (%)	7.2	7.0	8.3
Inter-Assay CV (%)	8.8	6.0	5.5
Spike Test* (%)	94	92	100

*Known amounts of mouse aggrecan were added to samples and then diluted with Sample/Standard Dilution Buffer (Solution C) to assay mouse aggrecan by ELISA.

TROUBLESHOOTING

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

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