Glycosaminoglycans Assay Kit

Catalog # 6022

For Research Use Only - Not Human or Therapeutic Use

PRODUCT SPECIFICATIONS

DESCRIPTION:	Assay kit to quantify GAGs
FORMAT:	96-well ELISA plate with removeable strips
ASSAY TYPE:	Colorimetric assay
ASSAY TIME:	5 minutes
STANDARD RANGE:	50 µg/ml to 3.1 µg/ml
NUMBER OF SAMPLES:	Samples NOT containing extra proteins: up to 42 (duplicate) samples/plate
	Samples containing extra proteins: up to 21 (duplicate) samples/plate
SAMPLE TYPES:	Tissue homogenate
RECOMMENDED SAMPLE DILUTIONS:	Varies
CHROMOGEN:	N/A (read at 525 nm)
STORAGE:	-20°C for 12 months
VALIDATION DATA:	Intra-Assay (1.7-7.6%)/Inter-Assay (5.2-8%)/Spiking Test (106-117%)
NOTES:	This assay uses a 5-point standard curve
	Samples need to be solubilized before running assay

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INTRODUCTION

Glycosaminoglycans (GAGs) are negatively charged polysaccharides located in most connective tissues and extracellular matrices (ECM), as well as on the surfaces of many cell types. Consisting of repeating core disaccharide units, GAGs are categorized into four types: heparan/heparan sulfate, chondroitin/dermatan sulfate, keratin sulfate, and non-sulfated hyaluronan. Sulfated GAGs in the ECM exist as proteoglycans which typically consist of multiple glycosaminoglycan chains attached to a core protein (1). In a highly organized ECM, articular cartilage is composed of type II collagen, hyaluronan, link protein, and chondroitin sulfate-rich proteoglycans, which provide the osmotic resistance necessary for cartilage to resist compressive loads (2).

In addition to type II collagen, GAGs are considered an autoantigen of rheumatoid arthritis (RA), as anti-GAGs antibodies associated with ECM degradation exist in the serum of RA patients (3). Although the role of proteoglycans in RA is still unknown, research shows that immunizing mice with proteoglycans consisting of GAGs can induce arthritis (4). Similarly, the loss of ECM is the first observable change in osteoarthritis (OA), therefore, determining GAGs levels may be a useful marker of disease progression. Furthermore, the successful use of artificial cartilage for the treatment of OA necessitates the analysis of type II collagen and GAGs content to ensure the cartilage quality (5).

Chondrex, Inc. provides a sulfated GAGs Assay Kit (Cat # 6022) using cationic dye 1,9 dimethylmethylene blue (DMB) which binds to highly charged sulfated GAGs, not including hyaluronan (6). This kit utilizes an improved DMB solution, minimizing interference with negatively charged contaminants such as DNA and RNA and uses chondroitin sulfate as a standard for the analysis of ECM in cartilage. Moreover, to analyze collagen coexisting with GAGs in cartilage, Chondrex, Inc. provides a variety of type and species-specific collagen detection kits and total collagen detection kits. For more information, please visit www.chondrex.com or contact us at support@chondrex.com.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Chondroitin-Sulfate Standard (60221)	1 vial	0.5 mg/ml, 0.5 ml	-20°C
1,9 Dimethylmethlyene Blue (DMB) Dye Solution (60222)	1 bottle	10 ml	-20°C
PBS (60223)	1 bottle	50 ml	-20°C
ELISA Plate	1 each	96-well (8-well strips x 12)	-20°C

ASSAY OUTLINE

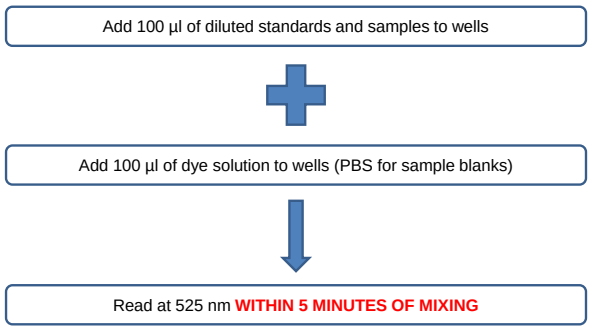
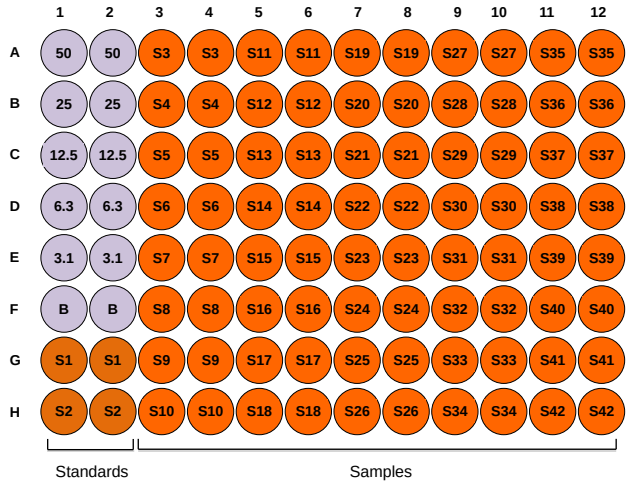
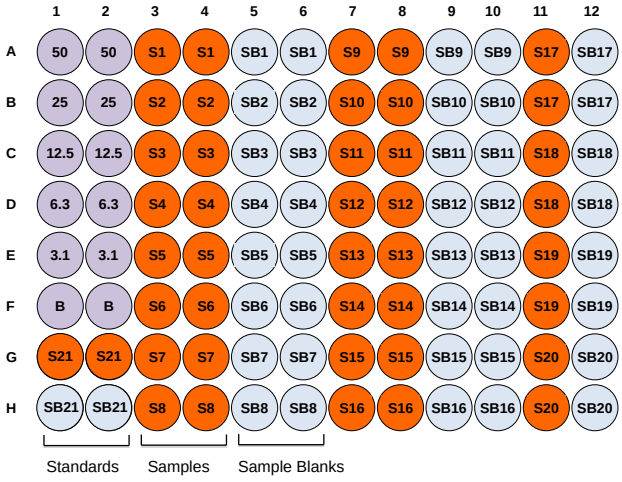


PLATE MAPPING

Assay Layout 1 - Samples NOT Containing Proteins



Assay Layout 2 - Samples Containing Proteins



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: Samples need to be solubilized. Please inquire at support@chondrex.com for Chondrex, Inc.'s "Tips on Glycosaminoglycan Solubilization"

NOTE 7: Guanidine used for extracting GAGs from samples will interfere with this assay. Guanidine extracted samples should be diluted or dialyzed against PBS to reduce the guanidine concentration to less than 0.25 M. In addition, some proteins have an absorbance at 525 nm; therefore, samples contaminated with unnecessary proteins must be diluted to a 5% or less protein solution and require sample blanks to ensure accurate results. Please refer to assay layout 2 for samples containing proteins.

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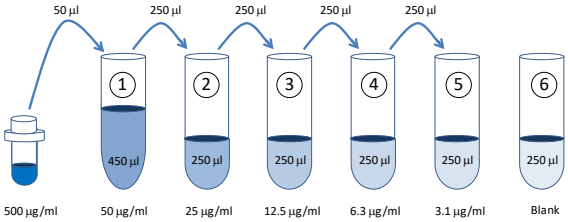
ASSAY PROCEDURE

1. **Prepare Standard Dilutions**: Take 50 µl of standard solution and mix with 450 µl of PBS (50 µg/ml). Then serially dilute the 50 µg/ml standard stock solution with PBS. For example, mix 250 µl of the 50 µg/ml standard stock solution with an equal volume of PBS to make a 25 µg/ml solution, and then repeat it three more times for 12.5, 6.3, and 3.1 µg/ml solutions. Chondrex, Inc. recommends making fresh serial dilutions for each assay.



2. **Prepare Sample dilutions**: Sample dilutions will vary depending on the source or preparation protocol of samples. Two to three different sample dilutions with PBS are recommended if the GAGs levels in the samples are unknown.

3. **Add Standards and Samples**: Choose 3-1 or 3-2 depending on your samples.

   3-1. Samples NOT containing extra proteins (use assay layout 1): Add 100 µl of standards, PBS (blank, B) into the purple wells, and samples into the orange wells in duplicate. For example, add 100 µl of sample 1 into the S1 wells, and then add 100 µl of sample 2 into the S2 wells. Proceed to Step 4-1.

   3-2. Samples containing extra proteins (use assay layout 2): Add 100 µl of standards, PBS (blank, B) into the purple wells, and samples into the orange and gray wells (SB) in duplicate. For example, add 100 µl of sample 1 into the S1 and SB1 wells, and then add 100 µl of sample 2 into the S2 and SB2 wells. Proceed to Step 4-2.

4. **Add Dye Solution**: Choose 4-1 or 4-2 depending on your samples.

   4-1. Samples NOT containing extra proteins (use assay layout 1): Add 100 µl of Dye Solution into all wells.

   4-2. Samples containing extra proteins (use assay layout 2): Add 100 µl of Dye Solution into the purple and orange wells and add 100 µl of PBS into the gray wells (SB).

5. **Read Plate**: Read the plate at 525 nm (or 530 nm) within 5 minutes after performing step 4.

CALCULATING RESULTS

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

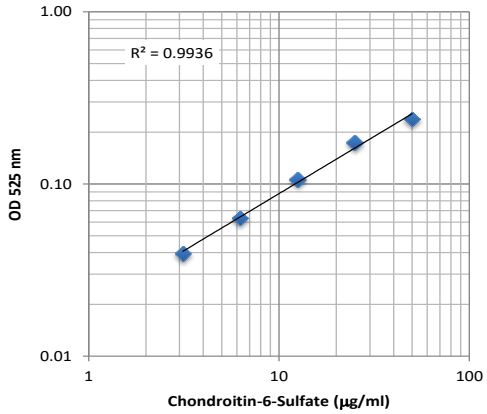
2. Subtract the averaged blank OD values from the averaged OD values of the standards and test samples.

   NOTE: If sample blanks are used, subtract averaged "sample blank" (SB) OD values from the averaged OD values of the corresponding test samples in Step 1

3. Plot the OD values of standards against the concentration of chondroitin-sulfate (µg/ml). Using a log/log plot will linearize the data. Figure 1 shows a representative experiment where the standard range is 3.1 - 50 µg/ml.

4. Chondroitin-sulfate concentration in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the chondroitin-sulfate concentration (µg/ml) in the original sample specimens. For additional assistance, please download a sample calculation worksheet from www.chondrex.com.

Figure 1 - A Typical Standard Curve for the Glycosaminoglycans Assay Kit



ASSAY VALIDATION

Table 1 - Reproducibility Data for the Glycosaminoglycans Assay Kit

Test	6.3 µg/ml	12.5 µg/ml	25 µg/ml
Intra-Assay CV (%)	7.6	3.6	1.7
Inter-Assay CV (%)	7.3	5.2	8.0
Spike Test* (%)	116%	117%	106%

*Known amounts of chondroitin-sulfate were added to standards and then diluted with PBS for assaying GAGs.

TROUBLESHOOTING

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

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6. I. Barbosa, S. Garcia, V. Barbier-Chassefière, J. Caruelle, I. Martelly, D. Papy-García, *et al*., Improved and Simple Micro Assay for Sulfated Glycosaminoglycans Quantification in Biological Extracts and Its Use in Skin and Muscle Tissue Studies. *Glycobiology* **13**, 647-53 (2003).