

Glycosaminoglycans Assay Kit

Catalog # 6022

For Research Use Only - Not Human or Therapeutic Use

INTRODUCTION

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

In addition to type II collagen, GAGs are considered to be an autoantigen of rheumatoid arthritis (RA), since anti-GAGs antibodies associated with ECM degradation exist in the sera from RA patients (3). In fact, immunization with proteoglycans consisted of GAGs can induce arthritis in mice (4); however, the pathogenesis of proteoglycan in RA is still under investigation. 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 provides a sulfated GAGs assay kit using the 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 chondroitin sulfate as an appropriate standard for the analysis of ECM in cartilage. Moreover, to analyze collagen coexisting with GAGs in cartilage, a variety of collagen detection kits is available, such as a type II collagen ELISA (catalog # 6018), type I collagen ELISAs (catalog # 6012, 6013, 6014, 6015, 6016, 6019, and 6021), a hydroxyproline assay kit (catalog # 6017), a Sirius Red/ Fast Green staining kit (catalog # 9046). For more information, please visit our web site or contact customer service.

KIT COMPONENTS

Item	Quantity	Amount	Storage
Chondroitin-6-Sulfate Standard, 60221	1 vial	0.5 mg/ml, 1ml	-20°C
1,9 Dimethylmethylene Blue (DMB) Dye Solution, 60222	1 bottle	10 ml	-20°C
PBS, 60223	1 bottle	50 ml	-20°C
ELISA Plate, 9026	1 plate	96-well	-20°C

Figure 1 - Standard Assay Layout 1

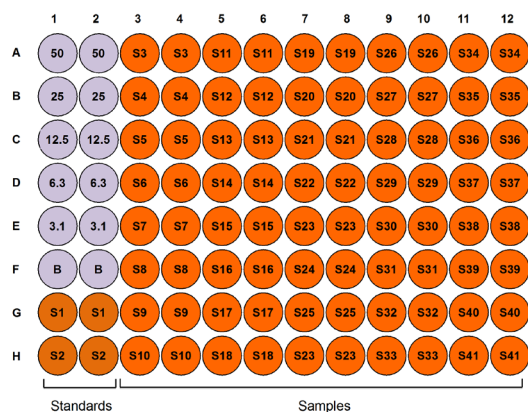
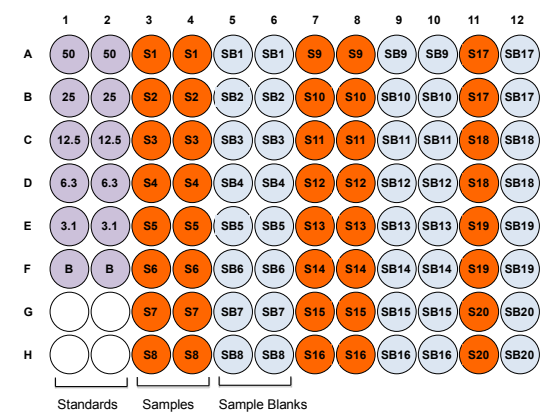


Figure 2 - Standard Assay Layout 2



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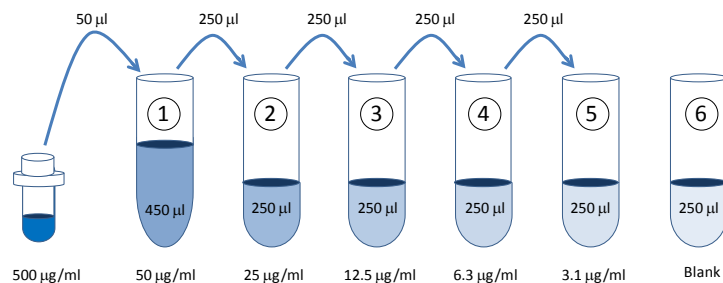
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NOTES BEFORE USING ASSAY

1. Samples have to be solubilized for this assay. Please refer to “Tips for the Solubilization of GAGs”.
2. It is recommended that the standards and samples be run in duplicate.
3. Assay Interference:
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 protein have to be diluted at less than 5% of protein, and require sample blanks to ensure accurate results. Please refer to assay layout 2 (Figure 2) for samples containing protein.

ASSAY PROCEDURE

1. **Prepare Standard Dilutions:** Take 50 μl of Standard solution and mix with 450 μl of PBS (50 $\mu\text{g}/\text{ml}$). Then serially dilute the 50 $\mu\text{g}/\text{ml}$ solution with Solution A. For example, mix 250 μl of the 50 $\mu\text{g}/\text{ml}$ solution with an equal volume of Solution A to make a 25 $\mu\text{g}/\text{ml}$ solution, and then repeat it three more times for 12.5, 6.3, and 3.1 $\mu\text{g}/\text{ml}$ solutions. We recommend making fresh serial dilutions for each assay.



2. **Prepare Sample dilutions:** The dilution of samples varies depending on the source or preparation protocol of samples. Dilute samples with PBS to fit within the standard range.
3. **Add Standards and Samples:** Choose 3-1 or 3-2 depending on your samples.
 - 3-1. Samples NOT containing proteins:
Use the plate layout as shown in Figure 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 proteins:
Use the plate layout as shown in Figure 2. Add 100 μl of standards, PBS (blank, B) into the purple wells, and samples into the orange and blue wells in duplicate. For example, add 100 μl of sample 1 into the S1 and SB1 wells, 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 proteins:
Add 100 μl of Dye Solution into all wells in Figure 1.
 - 4-2. Samples containing proteins:
Add 100 μl of Dye Solution into the purple and orange wells, and add 100 μl of PBS into the blue wells in Figure 2.
5. **Read Plate:** Read the plate at 525 nm (or 530 nm) within 5 minutes after step 4.

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CALCULATION OF GAGs CONCENTRATION

1. Average the duplicate OD values for the blank, (and sample blank), standards, and test samples.
2. Subtract the “blank” (B) values from the averaged OD values of standards and test samples in step 1.

Note: If sample blanks are used, subtract “sample blank (SB) 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-6-sulfate (µg/ml). Using a log/log plot will linearize the data. Figure 3 shows a representative experiment where the standard range is from 3.1 - 50 µg/ml.
4. The µg/ml of GAGs in test samples can be calculated using regression analysis. Multiply it by the sample dilution factor to obtain the GAG concentration (µg/ml) in the original sample specimen. Visit www.chondrex.com to download a calculation template for the GAGs assay kit, <http://www.chondrex.com/products/glycosaminoglycans-assay-kit>.

Figure 3 - Typical Standard Curve for GAGs Assay

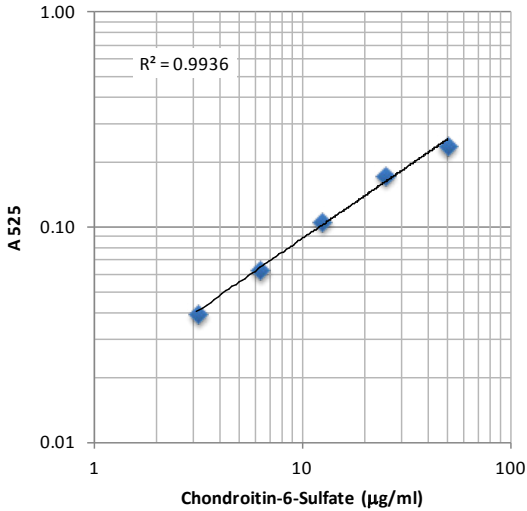


Table 1 - Reproducibility of Data Assayed by the GAGs Assay Kit

Test At	6.3 µg/ml	12.5 µg/ml	25 µg/ml
Inter-Assay CV (%)	7.3	5.2	8.0
Intra-Assay CV (%)	7.6	3.6	1.7
Spiking Test*	115.1%	116.2%	105.1%

Standard was added with known amounts of chondroitin-6-sulfate and then diluted with Sample/Standard Dilution Buffer for assaying GAGs.

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