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Sulfated-Glycosaminoglycans Assay Kit

rev 04/21

(Catalog # K2074-100; 100 assays, Store kit at -20 °C)

I. Introduction:

Glycosaminoglycans (GAGs) or mucopolysaccharides are long, linear, negatively charged polysaccharides consisting of repeating disaccharide units. Based on its core disaccharide structure, GAGs are categorized into four groups, sulfated GAGs including heparin/heparan sulfate, chondroitin sulfate, keratan sulfate and non-sulfated GAGs including hyaluronic acid, respectively. They are the most abundant heteropolysaccharides in the human eye. They are a key constituent of the extracellular matrix and act as a filler substance between cells and fibers in tissues. They vary in molecular mass (10-100 kDa), disaccharide construction, sulfation and have diverse functions. GAGs are the structural scaffolds and provide support and adhesiveness in bone, corneas, skin and connective tissues. Additionally, GAGs play an important role in many biological processes including cell signaling, cell growth and proliferation, anti-coagulation, angiogenesis, tumor progression, etc. GAGs are degraded in the lysosome via four different pathways. However, patients with the lysosomal storage disorder known as mucopolysaccharidoses (MPSs) have impaired lysosomal break down of GAGs and hence have higher levels of GAGs in their tissues or urine. Abnormal accumulation of GAGs in cells, blood and connective tissues over time can lead to permanent cell damage that can affect appearance, movement and functioning of organs. **BioVision's Sulfated-Glycosaminoglycans Assay Kit** efficiently detects all sulfated GAGs including heparan sulfate, chondroitin sulfate and keratan sulfate in various biological samples. In this assay, a cationic dye, 1,9-dimethylmethlene Blue (DMMB) specifically interacts with the highly negatively charged sulfated GAGs to produce a colored product, which is measured at absorbance 525 nm. The colored signal is directly proportional to the concentration of sulfated GAGs in the sample. The kit offers a simple, rapid, sensitive and convenient way to measure the amount of sulfated GAGs in samples. It can detect as low as 0.5 µg of sulfated GAGs under the assay conditions.

Sulfated Glycosaminoglycan (S-GAG) $\xrightarrow{\text{DMMB}}$ **Colored Product (OD 525 nm)**

II. Application:

- Determination of sulfated GAGs in biological samples.

III. Sample Type:

- Urine
- Tissue sample such as muscle

IV. Kit Contents:

Components	K2074-100	Cap Code	Part Number
S-GAG Assay Buffer	20 ml	WM	K2074-100-1
S-GAG Standard	1 ml	Yellow	K2074-100-2
S-GAG Dye	20 ml	Amber	K2074-100-3

V. User Supplied Reagents and Equipment:

- Homogenization Buffer (PBS with 0.5% Tergitol)
- Dounce Tissue Homogenizer (BioVision Cat. 1998)
- Multi-well spectrophotometer
- 96-well clear flat-bottom plate
- Multichannel pipette

VI. Storage Conditions and Reagent Preparations:

Store the kit at -20 °C. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

- **S-GAG Assay Buffer:** Ready to use. Warm to Room Temperature (RT) before use. Store at 4 °C.
- **S-GAG Standard (1 mg/ml) and S-GAG Dye:** Ready to use. Warm to RT before use. Store at -20 °C.

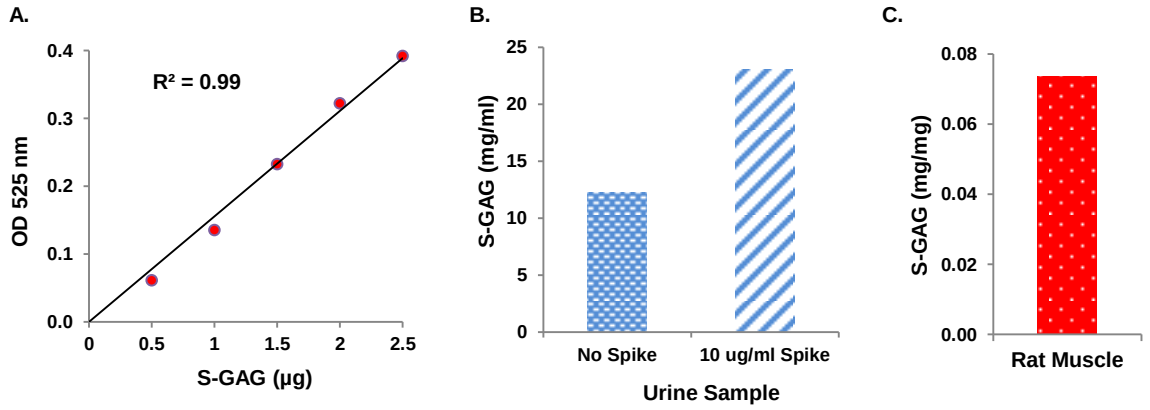
VII. Sulfated Glycosaminoglycans Assay Protocol:

1. **Sample Preparation:** Transfer ~100 mg of muscle tissue in an eppendorf tube. Add 2X volume of ice cold Homogenization Buffer to the tube and gently homogenize the tissue using Dounce Tissue Homogenizer (BioVision Cat. 1998). Centrifuge the sample at 12,000 x *g* and 4 °C for 20 min and collect the supernatant for the assay. For each sample type, add 50 µl of the supernatant into a well of a 96-well clear, flat bottom plate labeled as **Sample**. Add 50 µl of the Homogenization Buffer into another well labeled as **Sample Control**. Adjust the volume of Sample and Sample Control well to 100 µl using S-GAG Assay Buffer.
   For urine sample: Centrifuge 500 µl of urine sample in an eppendorf tube at 12,000 x *g* for 10 min at RT and collect the supernatant. Add 100 µl of the supernatant into a well of a clear, flat bottom 96-well plate labeled as **Sample**.
2. **Standard Curve Preparation:** Prepare 0.1 mg/ml of diluted S-GAG Standard by mixing 100 µl of the S-GAG stock Standard with 900 µl of S-GAG Assay Buffer. Add 0, 5, 10, 15, 20 and 25 µl of diluted S-GAG Standard into the desired wells to generate 0, 0.5, 1, 1.5, 2 and 2.5 µg of S-GAG Standard/well respectively. Adjust the volume of each well to 100 µl with S-GAG Assay Buffer.
3. **Reagent Addition:** Use a multichannel pipette, add 200 µl of S-GAG Dye to all wells including S-GAG Standard, Sample(s), Sample Control. Incubate the plate for 2 min at RT.

4. **Measurement:** Measure the absorbance of all wells at 525 nm in end-point mode at RT.
5. **Calculation:** Subtract the 0 Standard reading from all Standard readings and Sample Control reading from all Sample readings respectively. Plot the S-GAG Standard Curve. Apply the corrected Sample readings to the S-GAG Standard Curve to get A µg of Sulfated-Glycosaminoglycans in the samples.

Sulfated-Glycosaminoglycans in sample(s) (µg/ml) = A x D/V

Where: A = Amount of S-GAG from the Standard Curve (µg)
D = Sample dilution factor (D = 1 for undiluted samples)
V = Volume of sample used (ml)



Figures. A. S-GAG Standard Curve. **B.** Concentration of S-GAGs in unspiked and spiked urine samples. > 95% recovery was observed when spiked with 10 µg/ml S-GAG. **C**. Amount of S-GAGs in rat muscles. Assay was performed according to the assay protocol.

IX. Related Products:

Urea Colorimetric Assay Kit (K375)
Urease Activity Assay Kit (Colorimetric) (K378)
Aspartate Colorimetric/Fluorometric Assay Kit (K552)
Urea Colorimetric Assay Kit II (K376)
Arginine Assay Kit (Fluorometric) (K384)
Citrulline Fluorometric Assay Kit (K2002)

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