



Total Glycosaminoglycans Assay Kit (Colorimetric)

(Catalog # K2085-100; 100 assays, Store kit at 4 °C)

04/21

I. Introduction:

Glycosaminoglycans (GAGs) or mucopolysaccharides are long, linear, negatively charged polysaccharides consisting of repeating disaccharide units. Based on their 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, anticoagulation, 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 Total Glycosaminoglycans Assay Kit can measure the total amount of GAGs including sulfated GAGs such as chondroitin sulfate, heparan sulfate etc. and non-sulfated GAGs such as hyaluronic acid in various biological samples. In this assay, GAGs interacts with a specific probe to form a colored product that is measured by absorbance at 400 nm. The colored signal is directly proportional to the GAG concentration in the sample. The kit offers a simple, rapid, sensitive and convenient way to measure the total amount of GAGs in various sample types. It can detect as low as 1 µg of chondroitin sulfate or hyaluronic acid under the assay conditions.



II. Application:

• Determination of GAGs in various sample types.

III. Sample Types:

- Saliva
- Tissue samples (i.e. brain and muscle)
- Foods and drinks

IV. Kit Contents:

Components	K2085-100	Cap Code	Part Number
GAG Assay Buffer GAG Standard (1 mg/ml)	25 ml 4 ml	WM NM	K2085-100-1 K2085-100-2
GAG Probe	25 ml	NM	K2085-100-3

V. User Supplied Reagents and Equipment:

- 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 4 °C. The kit components are stable for one year when stored as recommended. Read the entire protocol before performing the experiment.

• GAG Assay Buffer, GAG Standard & GAG Probe: Ready to use. Warm to Room Temperature (RT) before use. Store at 4 °C.

VII. Glycosaminoglycans Assay Protocol:

- 1. Sample Preparation: Liquid samples (i.e. saliva and orange juice): Liquid samples are centrifuged at 12,000 x g and 4 °C for 20 min and the supernatant is collected for the assay. Solid samples (i.e. banana or tissue samples): Cut the solid samples into small pieces and transfer ~200 mg of sample into an eppendorf tube. Add 2X volume of ice-cold GAG Assay Buffer to the tube and gently homogenize using a Dounce Tissue Homogenizer (BioVision Cat. 1998). Centrifuge the sample at 12,000 x g and 4 °C for 20 min and collect the supernatant. Add 10-50 µl of the supernatant into a well of a 96-well clear flat bottom plate labeled as Sample. Use the same volume of GAG Assay Buffer as the Background Control. Adjust the volume of each well to 100 µl using GAG Assay Buffer. Note: For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range.
- 2. Standard Curve Preparation: Add 0, 2, 4, 6, 8 and 10 µl of GAG Standard (1 mg/ml) into the desired wells of a 96-well clear flat bottom plate to generate 0, 2, 4, 6, 8 and 10 µg of GAG Standard/well respectively. Adjust the volume of each well to 100 µl using GAG Assay Buffer.





- **3. Probe Addition:** Use a multichannel pipette to add 200 µl of GAG Probe to all wells including GAG Standard, Sample(s) and Background Control. Incubate the plate for 2 min at RT.
- 4. Measurement: Measure the absorbance of all wells at 400 nm in end-point mode at RT.
- 5. Calculation: Subtract the 0 Standard reading from all Standard readings and Background Control reading from all Sample readings respectively. Plot the GAG Standard Curve. Apply the corrected Sample readings to the GAG Standard Curve to get A µg of GAG in the samples.

Glycosaminoglycans in liquid sample(s) (µg/µl) = A x D/V

Where: A = Amount of GAG from the Standard Curve (µg)

D = Sample dilution factor (D = 1 for undiluted samples)

V = Volume of sample used (µI)

Glycosaminoglycans in solid sample(s) (µg/mg) = A x D x T /V x M



Figures. A. GAG Standard Curve. **B.** Amount of GAGs detected in saliva (50 µl) and orange juice (10 µl) samples. />. 90% recovery was observed when spiked with 2 µg of GAG. **C.** Amount of GAGs detected in banana and rat brain samples. Assay was performed according to the assay protocol.

VIII. Related Products:

Sulfated-Glycosaminoglycans Assay Kit (K2074) Urea Colorimetric Assay Kit (K375) Urease Activity Assay Kit (Colorimetric) (K378) Aspartate Colorimetric/Fluorometric Assay Kit (K552) Citrulline Fluorometric Assay Kit (K2002) Urea Colorimetric Assay Kit II (K376) Arginine Assay Kit (Fluorometric) (K384)

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