# QuantiChrom™ Glycosaminoglycans Assay Kit (DGAG-100)

**Semi-Quantitative Turbidimetric Assay for Glycosaminoglycans**

## DESCRIPTION

*GLYCOSAMINOGLYCANS (GAGs)* are long, linear polysaccharides and highly negatively charged molecules that constitute a major component of the extracellular matrix in mammalian tissues. GAGs play key roles in cell growth, cell adhesion, wound repair, and anticoagulation. They are also important for brain development and homeostasis. GAGs are associated with osteoarthritis, inflammation, diabetes, spinal cord injury, and cancer.

BioAssay Systems' GAGs assay uses a turbidimetric reaction in which GAGs form an insoluble complex with protein in the reagent. The increase in turbidity at 600nm is directly proportional to the glycosaminoglycans in the sample (reported in hyaluronic acid equivalents).

## KEY FEATURES

**Sensitive and accurate**. Use as little as 40 µL samples. Linear detection range in 96-well plate: 0.01 to 0.5 mg/mL hyaluronic acid.

**Fast and convenient**. The procedure involves addition of a single working reagent and incubation for 10 min. Room temperature assay. No 37°C incubator is needed.

**High-throughput**. Homogeneous "mix-incubate-measure" type assay. Can be readily automated to assay thousands of samples per day.

## APPLICATIONS

For quantitative determination of glycosaminoglycans in food and tissue homogenate.

## KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

**Reagent:** 16 mL **Standard:** 0.8 mL (0.5 mg/mL Hyaluronic Acid)

**Storage conditions**. The kit is shipped at RT. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

**Precautions**: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

## PROCEDURES

*Limitations*: 1. As with many turbidimetric assays, many factors, such as sample matrix components, non-specific binding, sensitivity to temperature and mixing may affect the reaction kinetics. We strongly recommend that a standard curve be run with each assay and all samples to be compared are run within the same assay. 2. Different types of GAGs may result in different signal strengths and standard curve shapes. This assay is reported in hyaluronic acid equivalents using hyaluronic acid as the GAGs standard.

### Sample Preparation

*Tissue and other solid samples (e.g. food)*: Prior to dissection, rinse tissue samples in 1X PBS (pH 7.4) to remove blood. Add sample and ice-cold 1X PBS (pH 7.4) to Dounce homogenizer at a ratio of 7.5 µL/mg tissue. We recommend using at least 15 mg of sample and keeping the mass similar across samples, if possible. Homogenize sample using 20 - 40 strokes with the pestle (stroke number will depend on how the sample type is; keep stroke number consistent across all samples in an experiment). Transfer homogenate and any remaining solids to a fresh 1.5-mL microcentrifuge tube. Rinse pestle and sides of homogenizer with an equal volume of PBS to the amount originally added; combine this rinse liquid with the homogenate. Sonicate for 90 seconds on ice. Centrifuge at 14,000 rpm for 5 min. Remove supernatant for assay.

*Note*: Anionic substances (e.g. SDS, sodium deoxycholate, etc.) will interfere with the assay and should be avoided in sample preparation. Some biological samples such as serum, plasma, urine, and CSF contain levels of GAGs which are too low to be detected using this assay. Some sample types such as tissue will require a sample blank (see Procedure for details).

For unknown samples, it is prudent to test several dilutions to determine an optimal sample dilution factor *n*.

**Reagent Preparation:** Equilibrate all reagents to 25°C. Briefly centrifuge tubes before use.

### Procedure using 96-well plate

1. *Standards*. Dilute standard in separate wells of a clear flat-bottom 96-well plate as described in the Table.

| No | Standard + $H_2O$ | HA (mg/mL) |
|---|---|---|
| 1 | 40 µL + 0 µL | 0.5 |
| 2 | 32 µL + 8 µL | 0.4 |
| 3 | 24 µL + 16 µL | 0.3 |
| 4 | 16 µL + 24 µL | 0.2 |
| 5 | 8 µL + 32 µL | 0.1 |
| 6 | 0 µL + 40 µL | 0 |

2. Transfer 40 µL of each sample into separate wells.

   *IMPORTANT*: A sample blank is required for tissue and potentially other sample types. In this case, transfer an additional 40 µL sample into a separate well as a sample blank.

3. Add 160 µL Reagent to all standard and sample wells.

   *If there sample blank wells are used, add 160 µL H2O to sample blank wells.*

   Tap plate to *mix briefly and thoroughly*.

4. Incubate at room temperature for 10 min. Use a plate reader to read $OD_{600nm}$.

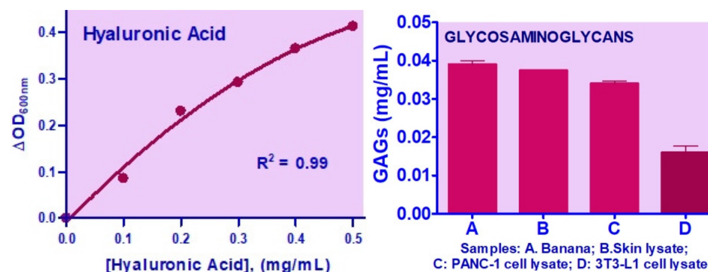
## CALCULATION

Subtract blank value (water, #6) from the standard OD values and plot the ΔOD against standard concentrations. Use a nonlinear fit for the standard curve, such as a $2^{nd}$ order polynomial equation. Subtract the blank OD value from sample well OD values. *(If sample blanks were used, instead subtract the sample blank OD values from samples.)* Use the standard curve equation to determine the sample GAG concentration. If sample was diluted prior to assay, multiply the result by the dilution factor.

*Note*: if the calculated concentration is above 0.5 mg/mL, dilute sample in water and repeat assay. Multiple the result by the dilution factor.

## MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader. If solid sample preparation is required: 1X PBS (pH 7.4), Dounce homogenizer, sonicator, centrifuge.




*Left*: Hyaluronic acid standard curve. *Right*: Samples were treated as described and assayed in the protocol

## LITERATURE

1. Köwitsch A. et al (2018) Medical application of glycosaminoglycans: a review. J Tissue Eng Regen Med. 12(1):e23-e41.
2. Dong J. et al (2024) Metabolism mechanism of glycosaminoglycans by the gut microbiota: Bacteroides and lactic acid bacteria: A review. Carbohydr Polym. 332:121905.
3. Wieboldt R, Läubli H. (2022) Glycosaminoglycans in cancer therapy. Am J Physiol Cell Physiol. 322(6):C1187-C1200.

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