

Technical Bulletin

Glycosaminoglycans Assay Kit

Catalogue Number MAK611

Product Description

Glycosaminoglycans (GAGs) are long, linear, highly negatively charged polysaccharides that are major components of the extracellular matrix in mammalian tissues. GAGs play key roles in cell growth, adhesion, wound repair, anticoagulation, and brain development/homeostasis, and are associated with osteoarthritis, inflammation, diabetes, spinal cord injury, and cancer.

The Glycosaminoglycans Assay Kit uses a turbidimetric reaction in which GAGs form an insoluble complex with protein in the reagent. The increase in turbidity at 600 nm is directly proportional to the glycosaminoglycans in the sample and is reported in hyaluronic acid equivalents. The linear detection range is 0.01 to 0.5 mg/mL hyaluronic acid.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- Reagent 16 mL
Catalogue Number MAK611A
- Standard 1 vial
Catalogue Number MAK611B

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Spectrophotometric multi-well plate reader capable of reading at 600 nm
- Clear flat bottom 96-well plates. Cell culture or tissue culture treated plates

are not recommended. (Catalogue number M2936 or equivalent)

- 1.5 mL microcentrifuge tubes

For Solid sample preparation:

- 1X PBS (7.4 pH) (Catalogue number PPB006 or equivalent)
- Dounce homogenizer
- Sonicator
- Centrifuge

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The product is shipped at ambient temperature. Store kit at -20°C upon receipt.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Sample Preparation

Tissue and other solid samples:

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 .

Procedure

All samples and standards should be run in at least duplicates. Prior to assay, bring the reagents to room temperature.

Note: 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. It is strongly recommended that a standard curve be run with each assay and all samples to be compared are run within the same assay.

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.

Preparation of Standards

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

Table 1.
Standard Preparation

Std #	Standard (μL)	Purified water (μL)	HA Conc. (mg/mL)
1	40	0	0.5
2	32	8	0.4
3	24	16	0.3
4	16	24	0.2
5	8	32	0.1
6	0	40	0

Assay Reaction

1. 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.

2. Add 160 μL Reagent to all standard and sample wells. If the sample blank wells are used, add 160 μL H₂O to sample blank wells.
3. Tap the plate to mix briefly and thoroughly.
4. Incubate at room temperature for 10 minutes.
5. Use a plate reader to read the OD at 600nm.

Results

Calculations

1. Subtract the blank value (water, #6) from the standard OD values and plot the Δ OD against standard concentrations.
2. Use a nonlinear fit for the standard curve, such as a 2nd order polynomial equation.
3. Subtract the blank OD value from sample well OD values. (If sample blanks were used, instead subtract the sample blank OD values from samples.)
4. Use the standard curve equation to determine the sample GAG

concentration. If the 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.

Figure 1.
Exemplary standard curve

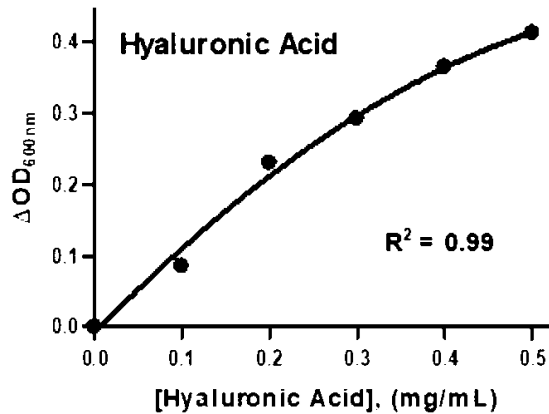
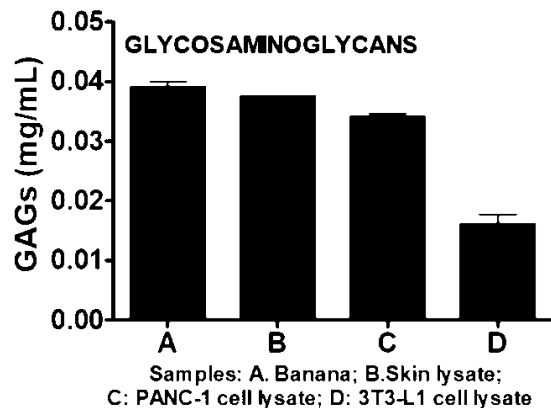


Figure 2.
Various samples tested



References

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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