



KAMIYA BIOMEDICAL COMPANY

sGAG Assay

**For the Quantitative Determination of Sulfated Glycosaminoglycans
in Synovial Fluid, Blood and Tissue Extracts**

Cat. No. BP-004

For Research Use Only. Not for Use in Diagnostic Procedures.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY®** sGAG Assay is a quantitative dye-binding assay for the *in vitro* analysis of sulfated glycosaminoglycans (sGAG). The assay is used to detect sGAGs in biological samples such as Synovial Fluid, Blood, and Tissue Extracts. For research use only. Not for use in diagnostic procedures.

DESCRIPTION

The most abundant heteropolysaccharides in the body are the glycosaminoglycans (GAGs). They are located primarily on the surface of cells or in the extracellular matrix. As an example, cartilage is composed to a large extent of GAGs, which are the dominant part of the proteoglycan Aggrecan.

GAGs are negatively charged long unbranched polymeric polysaccharides composed of repeating units of disaccharides containing one uronic acid or galactose and one amino sugar, either N-acetylglucosamine or N-acetylgalactosamine. The variation in charge may be very large since each disaccharide is more or less sulfated.

Based on GAG's high negative charge, a number of dye-binding procedures for their measurement have been developed. In most cases, however, they are not applicable to biological material without different forms of pre-treatment such as protease digestion. The present assay makes use of the dye Alcian Blue, which has a long history as a histological tissue staining reagent. Alcian Blue is a tetravalent cation with a hydrophobic core. The four charges allow the dye to bind to negatively charged polymers such as GAGs at high ionic strength, in contrast to other cationic dyes, which are all monovalent. The molecular structure of Alcian Blue, i.e. the plane tetragonal hydrophobic core with positive charges at its corners, may facilitate formation of aggregates of several molecules side by side rather than micelle formation. The ionic strength, pH and presence of detergents will affect the size of these aggregates in solution.

The ionic bonding between cationic dyes (such as Alcian Blue) and the negatively charged GAGs are generally thought to be proportional to the number of negative charges present on the GAG chain, i.e. both sulfated and carboxyl groups.

PRINCIPLE

The principle is based on the specific interaction between sulfated polymers and the tetravalent cationic dye Alcian Blue. The assay is performed at a pH low enough to neutralize all carboxylic and phosphoric acid groups and at an ionic strength large enough to eliminate ionic interactions other than those between Alcian Blue and sulfated GAGs. The Alcian Blue reagent may be obtained from a number of commercial sources. However, the quality of the reagent and its usability in proteoglycan assays differ dramatically from brand to brand. The Alcian Blue reagent in the **K-ASSAY®** sGAG Assay has been carefully selected and optimized for this particular use.

Hyaluronan, a non-sulfated GAG, does not react in this assay. There is no interference from proteins or nucleic acids in this method, in contrast to the DMMB-method or other dye binding methods.

COMPONENTS

- **10 mL Alcian Blue Stock Solution** containing 0.1% H₂SO₄ and 0.4M GuHCl. Dilute with SAT Solution.
- **10 mL GuHCl**, 8M Guanidine-HCl. Used to dilute samples.
- **65 mL SAT Solution** containing 0.3% H₂SO₄ and 0.75% Triton X-100. Used to dilute Alcian Blue Stock Solution and as addition to samples.
- **100 mL DMSO Solution** containing 40% dimethylsulfoxide and 0.05M MgCl₂. Used to wash pellets.
- **100 mL Gu-Prop Solution** containing 4M GuHCl, 33% 1-propanol and 0.25% Triton X-100. Used to dissolve pellets before reading the absorbance.
- **Six Calibrators** containing 1.1 mL of chondroitin sulfate-6 at 12.5, 25, 50, 100, 200, and 400 µg/mL diluted in water.
- **Control (Ctrl)** containing 1.1 mL of cartilage extract diluted in water.

STORAGE

All reagents should be stored at 4°C.

MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED

- Microplate reader or a spectrophotometer with 600-620 nm filter.
- Precision pipettes with disposable tips.
- Disposable syringe with 18 G needle for convenient removal of supernatants.
- Capped polypropylene vials (1.5 or 2 mL size) of Eppendorf type are recommended.
- Centrifuge capable of giving a centrifugal force of at least 12,000 x g.

SAMPLE PREPARATION

- GAGs are stable at 4°C in the absence of cells. The protein core of proteoglycans may be degraded by proteolytic activity in the sample which, however, does not alter the GAG chains.
- Cell debris and insoluble material should be removed by centrifugation (12,000 x g, 15 min).
- Sample volumes given below are only suggestive. The method is equally applicable at any scale as long as the final concentration of GuHCl is 0.4M at pH 1.5.
- The sGAG assay may be used with serum, plasma or synovial fluid. Handle as if capable of transmitting infectious agents.
- Store samples at 4°C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20°C or colder. Do not use a frost-free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield spurious results.
- The NCCLS provides recommendations for storing blood specimens, (Approved Standard Procedures for the Handling and Processing of Blood Specimens, H18A, 1990).

PROCEDURE

The procedure outlined here is valid for measuring sGAG in test tubes. Please inquire for Electrophoresis and dot blot assay instructions. All solutions should be used at room temperature.

Preparation of Dye Working Solution:

- Mix reagents with the following proportions to prepare Alcian Blue working solution:
50 mL SAT + 90 mL distilled water + 10 mL Alcian Blue Stock Solution.
- This volume is sufficient for 200 tests. The working solution is stable for 1 week at 4°C. Smaller volumes can be made using the same proportions.

Test Tube Procedure:

1. Pipette 50 µL/vial in duplicate of blank (BL, use water), calibrators (C), control (Ctrl) and samples (S) in test tubes according to the following layout.

BL	BL	C12.5	C12.5	C25	C25	C50	C50	C100	C100	C200	C200
C400	C400	Ctrl	Ctrl	S1	S1	S2	S2	etc	etc		

2. Add 50 µL of 8M GuHCl to each vial, mix and incubate at RT for 15 minutes.
3. Add 50 µL of SAT Solution to each vial, mix and incubate at RT for 15 minutes.
4. Add 750 µL of Alcian Blue working solution to each vial, mix and incubate at RT for at least 15 minutes. Alternatively, the samples can be incubated overnight at 4°C.
5. Centrifuge for 15 minutes at 12,000 x g.
6. Carefully remove supernatant by suction with a syringe and discard.
7. Add 500 µL of DMSO Solution to the pellet. Mix thoroughly. Make sure that the pellet becomes suspended. Mix for 15 minutes on a shaker at RT.
8. Centrifuge for 15 minutes at 12,000 x g.
9. Remove supernatant as above and discard.
10. Add 500 µL Gu-Prop Solution to the pellets. Mix for 15 minutes on a shaker. Check that the pellet is completely dissolved.

11. For each tube, dispense 240 μL /well into duplicate flat bottom microplate wells. Read the absorbance at 600-620 nm in an ELISA reader. Alternatively, absorbance can be read in a spectrophotometer at 600-620 nm. However, carryover between samples can be substantial, giving less accurate results.

CALCULATIONS

Plot absorbance values against the amount of GAG in each calibrator (12.5 - 400 $\mu\text{g/mL}$). The plot should be a straight line with an absorbance of approximately 2 at 400 $\mu\text{g/mL}$. Fit a linear equation to the data points and calculate the amount of GAG in each sample. Alternatively, if only a single calibrator concentration is used, a factor is calculated by dividing the calibrator concentration with the corresponding absorbance. The absorbance of the control and unknown sample is then multiplied with the factor to obtain the amount in each sample.

QUALITY CONTROL

The value of the control in this kit lot is found on the kit Certificate of Analysis.

NOTES

1. All commercial sulfated GAG samples (Cs, CsC, Ds, Ks, Hs) should give a similar color yield. Hyaluronan, DNA or RNA does not react with Alcian Blue under these conditions.
2. The sample must not contain any particles that sediment during centrifugation or is insoluble in 0.4M GuHCl at pH 1.5. If the sample does contain particles, it must be centrifuged before analysis and the supernatant used for analysis. If the sample contains material that precipitates in 0.4M GuHCl at pH 1.5, it must be removed by preceding preparative step.
3. The final concentration of GuHCl in the reagent mixture must be kept at 0.4M and the pH at 1.5-2.0.
4. Care should be taken that pellets are left intact when supernatants are removed by suction. The use of a manually operated syringe for suction and immediate removal of the supernatants after centrifugation is therefore recommended. The tube should be held at an angle and in such a way that the pellet and needle is visible during the entire operation. The needle should be gradually lowered with the meniscus during suction and never be allowed to touch the pellet.
5. Care should be taken that the pellets are completely dissolved in steps where this is required.

WARNINGS AND PRECAUTIONS

- For *in vitro* research use only
- The CDC and NIH recommend that potentially infectious agents be handled at the Biosafety Level 2.
- The kit contains a number of solutions that are corrosive/irritating chemical compounds (such as sulfuric acid, guanidine hydrochloride and DMSO). They should all be handled by knowledgeable persons with proper care and according to routine precautions/regulations for handling hazardous chemicals. Avoid swallowing and contact with the skin or mucous membranes. In case of contact with skin or eyes, irrigate thoroughly with water and seek medical attention immediately.
- Never pipette by mouth or allow reagents to come into contact with skin.

FOR RESEARCH USE ONLY

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