ab289842 – Total Glycosaminoglycans Assay Kit (Colorimetric)

For the measurement of the total amount of Glycosaminoglycans (GAGs) including sulfated and non-sulfated GAGs in saliva, tissue, food, and drinks.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289842

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, 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. This Total Glycosaminoglycans Assay Kit (ab289842, K2085) can measure the total amount of Glycosaminoglycans (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.

Storage and Stability

On receipt assay kit can be stored at 4°C. Kit components are stable for one year when stored as recommended.

Materials Supplied

Item	Quantity	Storage Condition
GAG Assay Buffer	25 mL	4°C
GAG Standard	4 mL	4°C
GAG Probe	25 mL	4°C

Materials Required, Not Supplied
These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- Dounce Tissue Homogenizer
- Multichannel pipette

Reagent Preparation

– Before using the kit, spin the tubes prior to opening.

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

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. Centrifuge the sample at 12,000 x g and 4°C for 20 min and collect the supernatant.

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.

Assay Protocol

1. Add 10-50 µL of the supernatant into a well of a 96-well clear flat bottom plate labeled as Sample.
2. Use the same volume of GAG Assay Buffer as the Background Control.
3. Adjust the volume of each well to 100 µL using GAG Assay Buffer.
   Δ Note: *For Unknown Samples, we recommend running several dilutions of the samples to ensure that the readings are within the Standard Curve range.*
4. 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.

Measurement

Measure the absorbance of all wells at 400 nm in end-point mode at RT.

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 (µL)

Glycosaminoglycans in solid sample(s) (µg/mg) = A x D x T / (V x M)
Where:
A = Amount of GAG from the Standard Curve (µg)
D = Sample dilution factor (D = 1 for undiluted samples)
V = Volume of sample used (µL)
T = Total volume of sample (µL)
M = Mass of sample (mg)

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)

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