



Acid Mucopolysaccharide Assay

Catalog # PMC-AK03-COS

For research use only
Not for diagnostic use

V012315

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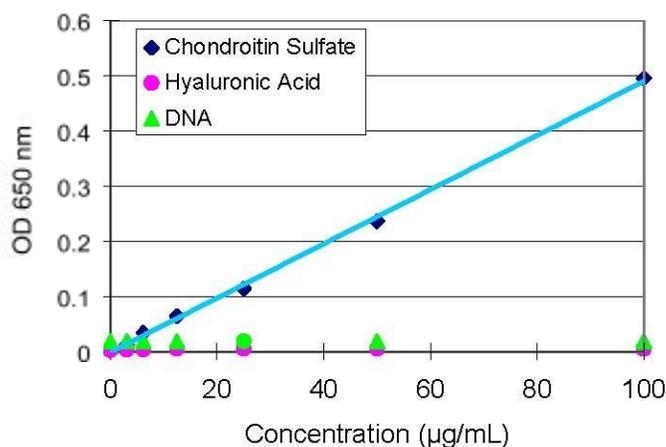
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Introduction

Mucopolysaccharides are a class of polysaccharides found in connective tissue that provides structural integrity to animals. Acid Mucopolysaccharide Assay is a quantitative colorimetric measurement of acidic mucopolysaccharide in animal cartilage tissue or cultured chondrocytes (cell layers or spheroid cell clusters). Cartilage tissue or cell layers are enzymatically treated to digest proteins and leaving behind the acidic mucopolysaccharides. The acidic mucopolysaccharides are stained and the resulting blue color is measured by a spectrophotometer at wavelength 650 nm.



Our Acid Mucopolysaccharide Assay uses the same buffer as DNA Quantity Assay and TRAP Staining Kit (PMC-AK04FN-COS), therefore a single sample can be shared among these assays.

Components

Store kit components at 4°C

- Stain Stock.....4 ml
- Buffer.....130 ml
- Enzyme Reagent, lyophilized5 vials
- Chondroitin Sulfate Standard (100 µg/ml).....2 ml

There are enough reagents to run 100 assays

Additional materials may be required

- Purified water
- 0.45 µm membrane filter
- Tubes
- Pipette
- Centrifuge
- Heater set at 60°C
- Spectrophotometer with 650 nm wavelength

Protocol

I. Reagent preparation and storage

1. Enzyme Solution
 - a. Dissolve 1 vial of lyophilized Enzyme Reagent in 10 ml of purified water.
 - b. Filter solution with a 0.45 μm membrane filter.
 - c. Aliquot and store the enzyme solution at -20°C until needed. The frozen enzyme solution is stable for 3 months. Avoid repeat freeze/thaw cycles.
2. Standard Solution
 - a. Serial dilute the Chondroitin Sulfate Standard (100 $\mu\text{g}/\text{ml}$) in sterile purified water to 100, 50, 25, 12.5, and 0 $\mu\text{g}/\text{ml}$.
 - b. The diluted standards can be stored frozen at -20°C for 1 year.
3. Stain Solution
 - a. Prepare Stain Solution just prior to performing the assay. Color of the diluted stain solution may fade in 5 seconds – 5 minutes. Fading is normal and does not affect the performance of the solution.
 - b. Bring the Staining Stock and Buffer to room temperature.
 - c. Mix 0.4 ml of Staining Stock to 12.6 ml of Buffer. Protect from light.

II. Cartilage Tissue Protocol

1. Add 10 ml of the Enzyme Solution to 1-10 mg of cartilage tissue. The amount of tissue depends on the amount of acidic mucopolysaccharide in the tissue.
2. Heat at 60°C for 1 hour to digest tissue completely
3. Pipet 100 μl of digested tissue or 100 μl of each Standard (100, 50, 25, 12.5, and 0 $\mu\text{g}/\text{ml}$) into a tube
4. Add 1.3 ml of Stain Solution to each tube
5. Mix thoroughly
6. A blue color will develop in a few minutes. Measure the absorption at 650 nm within 10 - 20 minutes of mixing.

Note: A high concentration (>120 $\mu\text{g}/\text{ml}$) of acidic mucopolysaccharides will precipitate. The sample must be diluted to obtain a concentration of less than 100 $\mu\text{g}/\text{ml}$.

III. Protocol for Cultured Cartilage Cells, Cell Monolayer or Spheroid Cell Clusters

1. Place cell layers or cell clusters into centrifuge tubes
2. Centrifuge at 1,500 rpm for 5 minutes
3. Remove the supernatant.
4. Add 0.5 ml of Enzyme Solution to the cell pellet
5. Digest the cells by heating at 60°C for 1 hour
6. Pipet 100 μl of digested sample or 100 μl of serial diluted Standards (100, 50, 25, 12.5, and 0

µg/ml) into a tube.

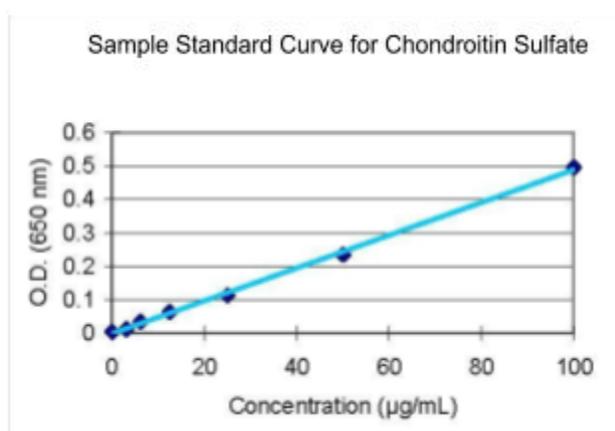
- Mix 1.3 ml of Stain Solution into each tube
- A blue color will develop in a few minutes. Measure the absorption at 650 nm within 10 - 20 minutes of mixing.

CALCULATIONS

- Plot the standard curve by graphing absorbance as a function of chondroitin sulfate concentration.
- Use the standard curve to determine mucopolysaccharide concentration

Note: If the sample has been diluted, the concentration obtained from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve



The chondroitin sulfate standard curve is provided for demonstration only. Standard curves should be generated for each set of samples assayed.

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