KAMIYA BIOMEDICAL COMPANY

Acidic Mucopolysaccharide Kit

For the quantitative colorimetric determination of Acidic Mucopolysaccharides in animal cartilage tissue or cultured chondrocytes

Cat. No. KT-007

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

PRODUCT INFORMATION

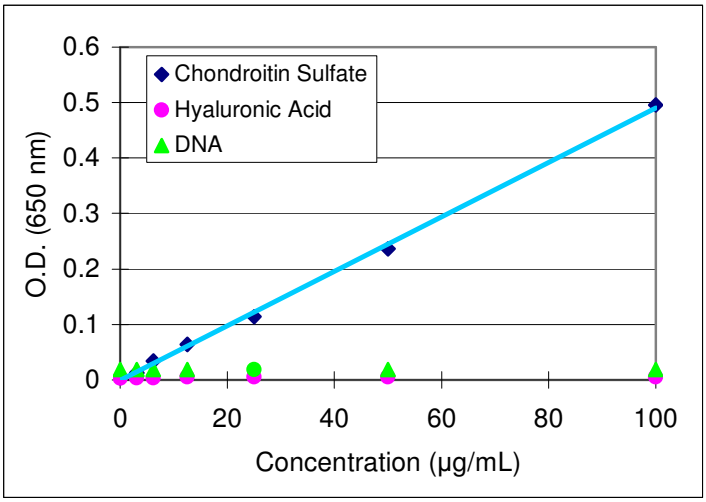
Acidic Mucopolysaccharide Kit
Cat. No. KT-007

PRINCIPLE

Mucopolysaccharides are a class of polysaccharides found in connective tissue that provides structural integrity to animals. The **K-ASSAY®** Acidic Mucopolysaccharide Kit is a simple and fast assay for the quantitative colorimetric determination of acidic mucopolysaccharides 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 leave behind the acidic mucopolysaccharides. The acidic mucopolysaccharides are stained and the resulting blue color is measured by a spectrophotometer at wavelength 650 nm. Conventional methodologies for the quantification of acidic mucopolysaccharides incorporate radioisotopes. Our colorimetric assay provides a convenient alternative to using radioisotopes. The Acidic Mucopolysaccharide Kit is for research use only. Not for use in diagnostic procedures.

The stain contained in the kit can differentiate between acid mucopolysaccharides and other acidic biological substances (see graph 1).

Graph 1



COMPONENTS

Reagent	Quantity
Staining Stock Solution	1 bottle, 4 mL
Buffer	1 bottle, 130 mL
Enzyme Reagent	5 vials, 10 mL/vial (lyophilized)
Chondroitin Sulfate Calibrator (100 µg/mL)	1 vial, 2 mL

There are enough reagents to run 100 assays.

Materials Required But Not Provided

Purified water
Spectrophotometer with 650 nm wavelength
Tubes
Centrifuge
Heater set at 60℃
0.45 µm membrane filter
Adjustable pipettor

STORAGE

Kit components can be stored at 4°C until expiration date.

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only, not for human or diagnostic use.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.

PROTOCOLS

Reagent Preparation

1. Enzyme Solution:
   a. Dissolve 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. Calibrator Solution:
   a. Serial dilute the Chondroitin Sulfate Calibrator (100 µg/mL) in sterile purified water to 100, 50, 25, 12.5, 6.3, and 3.1 µg/mL.
   b. The diluted calibrator can be stored frozen at -20°C for 1 year.

3. Staining Solution:
   a. Prepare Staining Stock Solution just prior to performing the assay. Color of the diluted staining stock solution may fade in 5 seconds - 5 minutes. Fading is normal and does not affect the performance of the solution.
   b. Mix 0.4 mL of Staining Stock Solution to 12.6 mL of Buffer. Protect from light.

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. Let cool.
4. Pipette 100 µL of digested tissue or 100 µL of each Calibrator (100, 50, 25, 12.5, 6.3, and 3.1 µg/mL) or 100 µL of water (blank) into a tube.
5. Add 1.3 mL of the Staining Stock Solution to each tube.
6. Mix thoroughly.
7. A blue color will develop in a few minutes. Measure the absorption at 650 nm within 20 minutes of mixing.

   *Note: A high concentration (>120 µg/mL) of acidic mucopolysaccharides will precipitate. The sample must be diluted to obtain a concentration of less than 100 µg/mL.

Cultured Cartilage Cells, Cell Monolayer or Spheroid Cell Clusters Protocol

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 the Enzyme Solution to the cell pellet.
5. Digest the cells by heating at 60°C for 1 hour.
6. Let cool.
7. Pipette 100 µL of digested sample or 100 µL of serial diluted Calibrator (100, 50, 25, 12.5, 6.3 and 3.1 µg/mL) or 100 µL of water (blank) into a tube.
8. Mix 1.3 mL of Staining Stock Solution into each tube.
9. Immediately measure the absorbance at 650 nm.

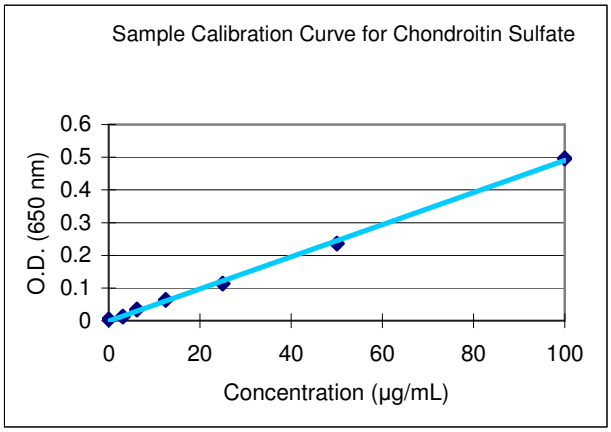
CALCULATIONS

1. Plot the calibration curve by graphing absorbance as a function of chondroitin sulfate concentration.
2. Use the calibration curve to determine mucopolysaccharide concentration.

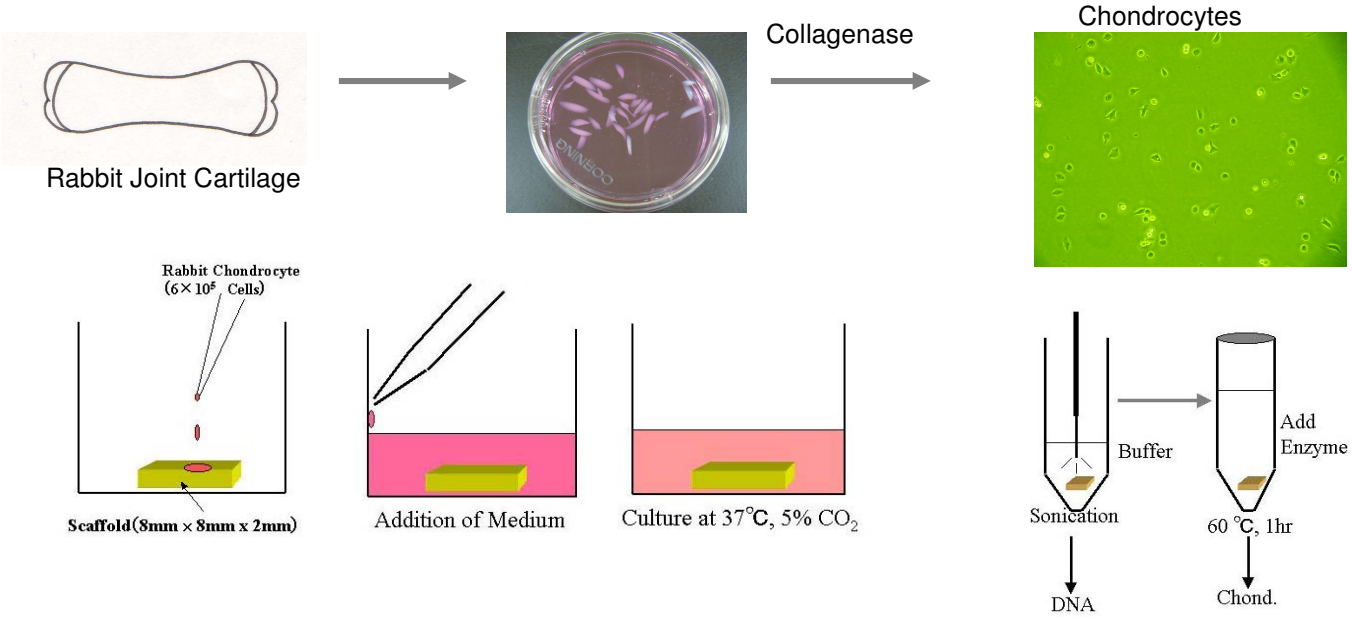
Note: If the sample has been diluted, the concentration obtained from the calibration curve must be multiplied by the dilution factor.

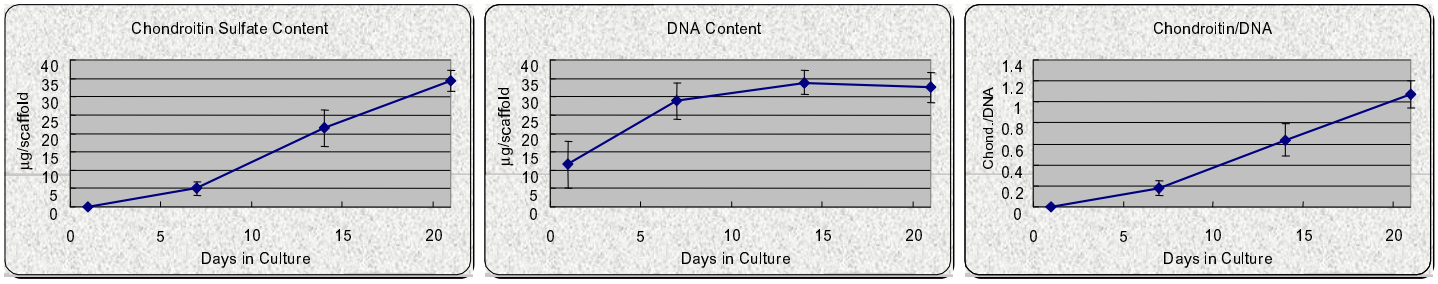
Typical Calibration Curve

Graph 2: The chondroitin sulfate calibration curve is provided for demonstration only. Calibration curves should be generated for each set of samples assayed.



Measurement of Chondroitin Sulfate and DNA content in a Scaffold for Cartilage Tissue Regeneration (used with DNA Quantification Kit, KT-045):





FOR RESEARCH USE ONLY
KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com