COSMO BIO CO., LTD.
Inspiration for Life Science

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Catalog No.PMC-AK03-COS

Acidic Mucopolysaccaride Assay Kit

Introduction

Chondrocytes produce extracellular matrix such as collagen, mucopolysaccharide and so on. There are conventional methods to measure acidic mucopolysaccharides using radioisotopes but the area and the amount used regarding radioisotopes are restricted. On the other hand, HPLC analysis is complicated to maintain the system and to extra for making samples. Acidic Mucopolysaccharide Assay Kit (PMC-AK03-COS) provides a convenient system for mucopolysaccharides visualization using Stains All, which combines with acidic mucopolysaccharides, with ease. Stains All normally combines with acidic substance, however the Stains All in this kit stains only acidic mucopolysaccharides of chondrocytes selectively.

Components/Storage

Components	Quantity	Stability
Staining Solution	4 mL	4-10ºC
Buffer	130 mL	4-10ºC
Enzyme Reagent	5 vials	4-10ºC
Standard Stock Solution (100 ug/mL Chondroitin Sulfate Calibrator)	2 mL	4-10ºC

One kit contains regents for 100 (20 samples × 5 times) samples

This kit cannot quantify various glycosaminoglycans individually.

This kit cannot quantify the mucopolysaccharides in culture medium.

Additional Materials Required

・distilled water

Prepare reagents

・Reaction Buffer

Warm Staining Solution and Buffer to room temperature. Mix 0.4 mL of Staining Solution and12.6 mL of Buffer.

Note: After mixing, the solution is blue but the color will fade in several minutes. This working solution cannot be stored.

・Standard Solution

Dilute the Standard Stock Solution in distilled water to 100, 50, 25, 12.5 and 0 ug/mL.

Note: This working solution can be stored frozen at -20°C.

・Enzyme Solution

Add 10 mL of distilled water to one vial of Enzyme Reagent and mix. Filter the solution with a membrane filter.

Note: This working solution cannot be stored.

Protocol

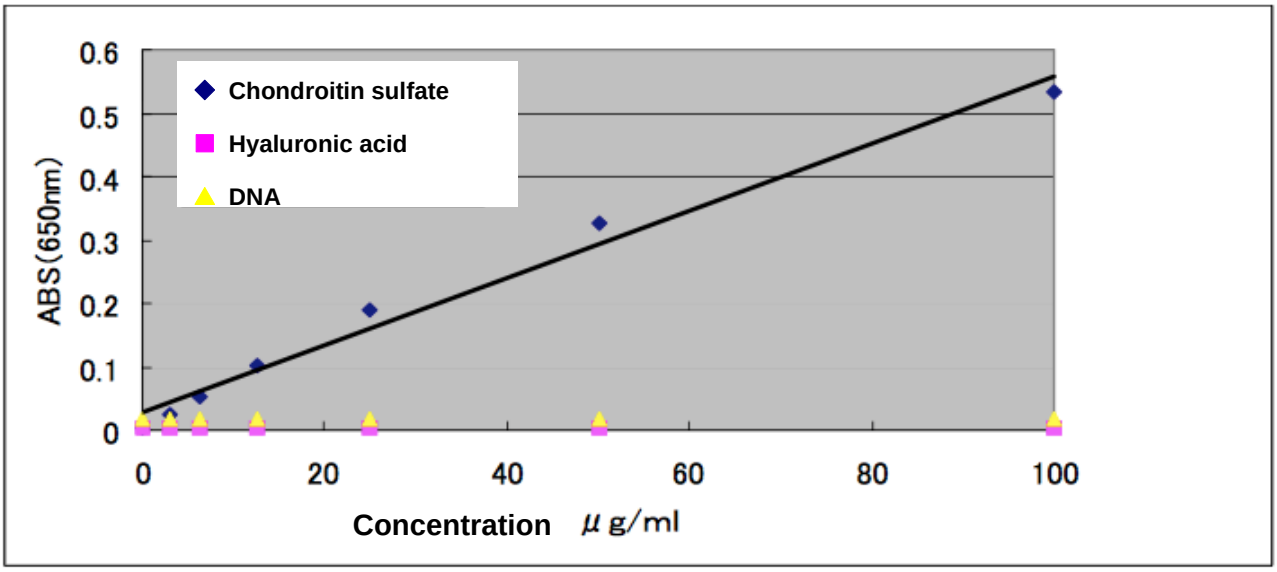
1. Prepare cultured chondrocyte layers or spheroid cell clusters.

Note: Using cell clusters is recommended. Refer to culture spheroid cell clusters method to know how to prepare spheroid cell clusters.

2. Remove culture medium and add 0.5 mL of Enzyme Solution. Digest the cells by incubating at 60°C for 1 hour.
3. Add 100 uL of digested cell samples or 100 uL of Standard Solution to tube, and add 1.3 mL of Reaction Buffer to each tube, and then leave the tubes.
4. The solution will become blue in a few minutes from the addition. Measure the absorption at 650 nm after the addition of 10 to 20 minutes.

Note: When the concentration of acidic mucopolysaccharides is more than 120 ug/mL, acidic mucopolysaccharides will precipitate. In the case, the sample must be diluted to obtain a concentration of less than 100 ug/mL. After the addition more than 20 minutes, acidic mucopolysaccharides will form blue precipitation

Fig.1 Acidic Mucopolysaccharide and acidic substances detected by Acidic Mucopolysaccharide Assay Kit
This kit stains only acidic mucopolysaccharides of chondrocytes selectively.



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Culture Spheroid Cell Clusters Method

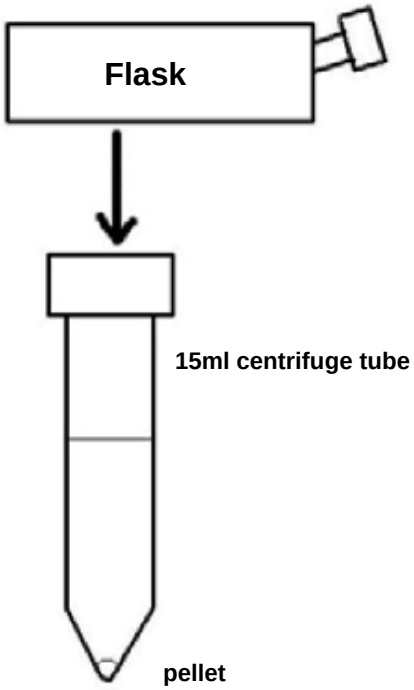

Fig.2 Cells form spheroid clusters under Hypoxia condition

Prepare cultured chondrocytes and cells are detached by the treatment of trypsin solution. Transfer detached cells to 15 mL centrifuge tube and centrifuge the centrifuge tube. Remove supplement and add 5 mL of medium to the centrifuge tube. In about 3 weeks, cells will form cartilage-like spheroids.

References

(1) Obayashi, K., Miyagawa-Tomita, S., Matsumoto, H., Koyama, H., Nakanishi, T., Hirose, H. Effects of Transforming Growth Factor-beta3 and Matrix Metalloproteinase-3 on The Pathogenesis of Chronic Mitral Valvular Disease in Dogs. Am. J. Vet. Res. 72, 194-202 (2011)

Notice to Purchaser

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