270695
MONOCLONAL ANTI CHONDROITIN - SULFATE
CLONE :
CS - 56

FORM :
Liquid

PRESERVATION :
0.1% sodium azide

PACKAGE SIZE :
0.5 ml processed ascites fluid

DESCRIPTION :
Monoclonal Anti Chondroitin - sulfate is a mouse IgM presented in the form of specially processed ascites fluid obtained from BALB / c mice bearing the CS - 56 hybridoma. This hybridoma is a cloned cell line derived from a fusion between a mouse myeloma cell line and splenocytes from BALB / c mice immunized with ventral membranes of chicken gizzard fibroblasts. The antibody producing clones were selected according to the criteria described in Avnur, z. and Geiger, B. , *Cell* **38**, 811 - 822 (1984) . Monoclonal anti chondroitin - surfate is a homogenous population of antibody molecules which may be used for immunocytochemical localization of native chondroitin - sulfate and for the study of the interelationships between proteoglycans and different extracellular matrix proteins in tissues and cultured cells.

USES :
Many cellular activities depend on the interaction of cells with surrounding extracellular matrix (ECM) . Most cells, in intact tissue and in culture, are attached to an ECM. Epithelial cells are associated with the basement membrane, fibroblastic cells are usually embedded in a pericellular mesh of fibrils and tissue culture cells usually grow on a substrate which is covered by various ECM components. Many studies in the last few years have indicated that the matrix or its various isolated components provide not only adhesive surfaces for cells to grow on but has far - reaching effects on the rate of cell growth, mobility, morphogenesis and differentiation. Within the ECM, several glycoproteins and proteoglycans were identified and it was proposed that the different constituents interact with each other in a rather complex fashion. Among the ECM constituents we may mention fibronectin, collagen, laminin and other "adhesive" proteins as well as heparin sulfate and chondroitin sulfate - containing proteoglycans. The poor antigenicity of proteoglycan, especially of their glycoasminoglycan (GAG) moieties rendered it difficult to localize these molecules in tissue and cell culture. Monoclonal anti chondroitin - sulfate is specific for the glyco - saminoglycan (GAG) portion of native chondroitin - sulfate proteoglycan (CSPG) therefore it can be used to detect CSPG and to study

its special distribution and relationships to specific cell - substrate contacts and to the cytoskeleton using immunofluorescence techniques. CS - 56 antibodies reacted specifically with chondroitin sulfate of both types A and C but not with type B (dermatan sulfate) . Moreover, affinity adsorption of CS - 56 antibodies on CS - A or CS - C (but not on CS - B) coated plates abolished comletely their capacity to stain cultured cells. The monoclonal nature of the product guarantees the continuous production of a constant titer anti chondroitin - sulfate antibody with the same specificity and chemical identity.

NITIBODEY PERFORMANCE :

SPECIFICITY :

Monoclonal anti chondroitin - sulfate stains specifically chondroitin - sulfate containing proteoglycans in cultured mamalian cells using indirect immunofluorescent labeling. The product reacts best in bovine mammary gland epithelial (BMGE) cells, however good immunofluorescent labeling may also be obtained with human, chicken and murine fibroblasts and tissues (Procedure attached) .

WORKING DILUTION :

at least 1 : 200 - The antibody titier was decermined by indirect immunofluorescent labeling of bovine mammary gland epithelial (BMGE) cells using FITC conjugated affinity purified anti - mouse Fab at dilutions 1 / 20 - 1 / 40. In order to obtain best results in different cell or tissue preparations, we recommend to determine optimal working dilutions by titration test.

STRAGE :

For long strage keep at -20°C. For continuous use keep at 4°C. Avoid repeated freeze - thaw.

NOTE :

Laboratory reagent - not to be administered to humans nor used for any drug purposes.

REFERENCES :

1. Avnur Z. , and Geiger, B. , *Exp. Cell Res*. **158**, 321 - 332 (1985) .
2. Hay, E. D. , *J. Cell Biol*. , **91**, 205 (1981) .
3. Aplin, J. D. and Hughes, R. C. , *Biochim. Biophys. Acta*. **694**, 375 (1982) .
4. Christner, J. E. , Caterson, B. , and Baker, J. R. , *J. Biol. Chem*. **255**, 7102 (1980) .
5. Grinnell, F. , *Int. Rev. Cytol*. , **53**, 65 (1978) .

Indirect Fluorescence Labeling of Culture Cells for Chondroitin - Sultate

A. **Materials**

1. Tissue culture chamber / slide, 4 - chamber.
2. Epithelial or fibroblast cells in DMEM medium + 10% Fetal calf Serum.
3. PBS containing 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$(solution A) .

4. 0.05% Triton X - 100 in solution A (solution B) .
5. 3% Paraformaldehyde in solution A (solution C) .
6. FITC labeled anti - mouse Fab affinity purified antibody (APA) .
7. Aqueous mounting medium.
8. Cover slips (24x50 mm) .

B. **Cell Growth and Fixation**

1. Collect the cells from tissue culture dish at a stage of almost confluency, wash with medium and seed on to chamber / slides. Seed 1 - $2x10^4$ cells per chamber and grow cells in incubator for 2 - 3 days. Do not change medium.
2. Remove chamber / slide from incubator, discard medium.
3. Dip into beaker containing solution A ; drain excess solution by touching the slide of the chamber / slide upside down onto filter paper.
4. Add dropwise solution C with a pasteur pipette to the slides. Add enough solution to cover the cell layer. Incubate 10' at room temperature.
5. Drain excess solution C and dip into beaker containing solution B for 1' at room temprature.
6. Dip three time (5' each) into solution A. Excess solution A is drained before moving to a new beaker.

C. **Indirect Immunofluorescence Labeling**

1. Dilute monoclonal antibody in Solution A to dilution 1 : 50, 1 : 200, 1 : 800 . Add enough diluted primary antibody to cover the cell layer and incubate chamber / slide for 60' with lid closed at room temperature.
2. Wash as in step 6.
3. Dilute FITC labeled anti - mouse Fab of affinity purified antibody to dilution 1 : 10 - 1 : 20. Add enough diluted conjugate to cover the cell layer. Incubate for 30' at room temperature.
4. Wash as in step 6.
5. Drain excess solution A and remove chamber / slide plastic top and glue from glass bases. Add immediately 4 drops of aqueous mounting medium and cover with cover slip.

D. **Remarks**

1. Aqueous mounting medium solidifies after 1 hour and enables stable storage of labeled slides if kept at 4°C in the dark.
2. The titer of the antibody is defined as the dilution in which a definite positive reaction can be observed.