

**HEPARAN SULFATE & CHONDROITIN SULFATE
IMMUNOSTAINING**

Note: The researcher should optimize the precise conditions for a particular assay.

Manual 1: Immunohistochemistry for heparan sulfate & chondroitin sulfate

Reagents	Antibodies to heparan sulfate :10E4, 3G10 Antibodies to chondroitin sulfate : LY111, CS-56, 1-B-5, 2-B-6, 3-B-3 GAGases : Heparitinase I(SKK #100704) (20mU/mL of sodium acetate buffer-3.3mM calcium chloride, pH7.0) Chondroitinase ABC protease free(SKK #100332) (1~5U/mL of 20mM Tris-HCl buffer, pH8.0)
Procedure	1. Prepare slides and controls. 2. Preincubate sections with reaction buffer of GAGase for 15 min at 37°C. 3. Incubate with GAGase for 1~2 hr at 37°C. Wash. 4. Block endogenous peroxidase with 0.3% H ₂ O ₂ methanol. 5. Incubate with 1% BSA in PBS for 1 hr at RT. 6. Incubate with anti-HS or anti-CS antibody for 1~2 hr at RT. Wash. 7. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hr at RT. Wash. 8. Incubate with HRP substrate. Wash. 9. Observe by microscopy.
Abbreviation	BSA: bovine serum albumin. PBS: phosphate buffered saline. RT: room temperature. FITC: fluorescein isothiocyanate.
References	Antibodies to heparan sulfate 1)David, G. et al. J. Cell Biol., 119, 961-975(1992) Antibodies to chondroitin sulfate 1)M.P. Mark et al. Develop. Biol., 133, 475-488(1989) 2)Fukatsu, T. et al. Br. J. Cancer, 57, 74-78(1987) 3)Sorrell, J.M. et al. J. Immunol., 140, 4263-4270(1988)

Manual 2: Western blot for heparan sulfate & chondroitin sulfate proteoglycan

Reagents	Antibody to heparan sulfate : 3G10 Antibodies to chondroitin sulfate : 1-B-5, 2-B-6, 3-B-3 GAGases : Heparitinase I(SKK #100704) (200mU/mL of sodium acetate buffer-3.3mM calcium chloride, pH7.0) Chondroitinase ABC protease free(SKK #100332) (1~5U/mL of 20mM Tris-HCl buffer, pH8.0) Membranes : PVDF membrane or nitrocellulose membrane
Procedure	1. Incubate partially purified proteoglycan fractions with GAGase for 1 hr at 37°C. ① Treat 1.5 μg of sample with 2 mU of Heparitinase I for heparan sulfate proteoglycan. ② Treat 1 μg of sample with 100 mU of Chondroitinase ABC for chondroitin sulfate proteoglycan. 2. Run samples on SDS-PAGE under reducing conditions. 3. Transfer it to membrane. 4. Blocking with 10% skim milk in PBS for 30 min at 37°C. 5. Incubate with anti-HS or anti-CS antibody for 1 hr at RT. Wash. 6. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hr at RT. Wash. 7. Incubate with HRP substrate. Wash.
Abbreviation	SDS: sodium dodecyl sulfate. PAGE: polyacrylamide gel electrophoresis.
References	Antibody to heparan sulfate 1)Bai, X.M. et al. J. Histochem. Cytochem., 42, 1043-1054(1994) Antibody to chondroitin sulfate 1)Yada T et al. J.Histochem. Cytochem., 44, 969-980(1996)

Manual 3: Immunoprecipitation for chondroitin sulfate proteoglycan

Reagents	Antibodies to chondroitin sulfate : 1-B-5, 2-B-6 GAGase : Chondroitinase ABC protease free(SKK #100332) (1~5U/mL of 100mM sodium acetate buffer, pH8.0 or 20mM Tris-HCl buffer, pH8.0)
Procedure	<ol style="list-style-type: none"> 1. Prepare ¹²⁵I proteoglycan fractions by Chloramin T method. 2. Incubate ¹²⁵I labelled proteoglycan fractions with GAGase for 1 hr at 37°C. (Treat 3 μg of sample with 100 mU of Chondroitinase ABC) 3. Incubate sample with normal mouse IgG and Protein G Sepharose for 1 hr at 4°C. 4. Remove Protein G Sepharose binding non-specific immune complexes and save supernatant. 5. Incubate supernatant with anti-CS antibody and new Protein G Sepharose for 3 hr at 4°C. Wash. 6. Boil Protein G Sepharose binding specific immune complexes with SDS-PAGE sample buffer for 5 min. 7. Save supernatant after centrifugation. 8. Run supernatant on SDS-PAGE under reducing conditions. 9. Place gel in direct contact with X-ray film and develop using manufacturer's instructions.
References	No available

Manual 4: Flow cytometry for heparan sulfate

Reagents	Antibodies to heparan sulfate : 10E4, 3G10 Cells : K-562, PLC/PRF/5 GAGase : Heparitinase I(SKK #100704) (50mU/mL of phosphate buffer saline, pH7.4(PBS))
Procedure	<ol style="list-style-type: none"> 1. Incubate 1 x 10⁶ cells with 100 μL of Heparitinase I or PBS for 20min at 37°C. Wash. 2. Incubate cells with anti-HS antibody for 30 min at 4°C. Wash. 3. Incubate cells with FITC conjugated F(ab)₂ fragment anti-mouse IgG or IgM for 30 min at 4°C. Wash. 4. Analyze using manufacturer's instructions.
References	No available

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