

## HEPARAN SULFATE & CHONDROITIN SULFATE IMMUNOSTAINING

### Immunohistochemistry for Heparan Sulfate and 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 (20 mU/ml of sodium acetate buffer-3.3 mM calcium chloride, pH 7.0)

Chondroitinase ABC Protease free (1-5 U/ml of 20 mM Tris-HCl buffer, pH 8.0)

#### Procedure

1. Prepare slides and controls.
2. Preincubate sections with reaction buffer of GAGase for 15 minutes at 37°C.
3. Incubate with GAGase for 1-2 hours at 37°C. Wash.
4. Block endogenous peroxidase with 0.3% H<sub>2</sub>O<sub>2</sub> methanol.
5. Incubate with 1% BSA in PBS for 1 hour at room temperature.
6. Incubate with anti-HS or anti-CS antibody for 1-2 hours at room temperature. Wash.
7. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hour at room temperature. Wash.
8. Incubate with HRP substrate. Wash.
9. Observe by microscopy.

#### References

##### **Antibodies to Heparan Sulfate**

1. David, G. et al. J. Cell Biol., 119, 961-975 (1992)

##### **Antibodies to Chondroitin Sulfate**

1. Mark, M.P. 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)

### Flow Cytometry for Heparan Sulfate

#### Reagents

##### **Antibody to Heparan Sulfate:**

10E4, 3G10

##### **Cells:**

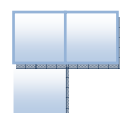
K-562, PLC/PRF/5

##### **GAGases:**

Heparitinase I (50 mU/ml of phosphate buffer saline, pH 7.4 (PBS))

#### Procedure

1. Incubate 1 x 10<sup>6</sup> cells with 100 µl of Heparitinase I or PBS for 20 minutes at 37°C. Wash.
2. Incubate cells with anti-HS for 30 minutes at 4°C. Wash.
3. Incubate cells with FITC conjugated F(ab')<sub>2</sub> fragment anti-mouse IgG or IgM for 30 minutes at 4°C. Wash.
4. Analyze using manufacturers instructions.



## Immunoprecipitation for Chondroitin Sulfate Proteoglycan

<b>Reagents</b>	<p><b>Antibodies to Chondroitin Sulfate:</b> 1-B-5, 2-B-6</p> <p><b>GAGases:</b> Chondroitinase ABC Protease free (1-5 U/ml of 100 mM sodium acetate buffer, pH 8.0 or 20 mM Tris-HCl buffer, pH 8.0)</p>
<b>Procedure</b>	<ol style="list-style-type: none"> <li>1. Prepare <sup>125</sup>I proteoglycan fractions by Chloramin T method.</li> <li>2. Incubate <sup>125</sup>I labeled proteoglycan fractions with GAGase for 1 hour at 37°C. (Treat 3 µg of sample with 100 mU of Chondroitinase ABC)</li> <li>3. Incubate sample with normal mouse IgG and Protein G Sepharose for 1 hour at 4°C.</li> <li>4. Remove Protein G Sepharose binding non-specific immune complexes and save supernatant.</li> <li>5. Incubate supernatant with anti-CS antibody and new Protein G Sepharose for 3 hours at 4°C. Wash.</li> <li>6. Boil Protein G Sepharose binding specific immune complexes with SDS-PAGE sample buffer for 5 minutes.</li> <li>7. Save supernatant after centrifugation.</li> <li>8. Run supernatant on SDS-PAGE under reducing conditions.</li> <li>9. Place gel in direct contact with X-ray film and develop using manufacturers instructions.</li> </ol>

## Western Blot for Heparan Sulfate Proteoglycan

<b>Reagents</b>	<p><b>Antibody to Heparan Sulfate:</b> 3G10</p> <p><b>GAGases:</b> Heparitinase I (200 mU/ml of sodium acetate buffer-3.3 mM calcium chloride, pH 7.0)</p> <p><b>Membranes:</b> PVDF membrane or nitrocellulose membrane</p>
<b>Procedure</b>	<ol style="list-style-type: none"> <li>1. Incubate partially purified proteoglycan fractions with GAGase for 1 hour at 37°C.             <ol style="list-style-type: none"> <li>a) Treat 1.5 µg of sample with 2 mU of Heparitinase I for heparan sulfate proteoglycan</li> <li>b) Treat 1 µg of sample with 100 mU of Chondroitinase ABC for chondroitin sulfate proteoglycan.</li> </ol> </li> <li>2. Run samples on SDS-PAGE under reducing conditions.</li> <li>3. Transfer it to membrane.</li> <li>4. Blocking with 10% skim milk in PBS for 30 minutes at 37°C.</li> <li>5. Incubate with anti-HS or anti-CS antibody for 1 hour at room temperature. Wash.</li> <li>6. Incubate with HRP conjugated anti-mouse IgG or IgM for 1 hour at room temperature. Wash.</li> <li>7. Incubate with HRP substrate. Wash.</li> </ol>
<b>References</b>	<p><b>Antibodies to Heparan Sulfate</b></p> <ol style="list-style-type: none"> <li>1. Bai, X.M. et al. J. Histochem. Cytochem., 42, 1043-1054 (1994)</li> </ol> <p><b>Antibodies to Chondroitin Sulfate</b></p> <ol style="list-style-type: none"> <li>1. Yada, T. et al. J. Histochem. Cytochem., 44, 969-980 (1996)</li> </ol>

### Abbreviations

BSA	- Bovine serum albumin	PBS	- Phosphate buffered saline
FITC	- Fluorescein isothiocyanate	HUVECs	- human umbilical vein endothelial cells
SDS	- Sodium dodecyl sulfate	PAGE	- polyacrylamide gel electrophoresis

