Programs of Excellence in Glycosciences

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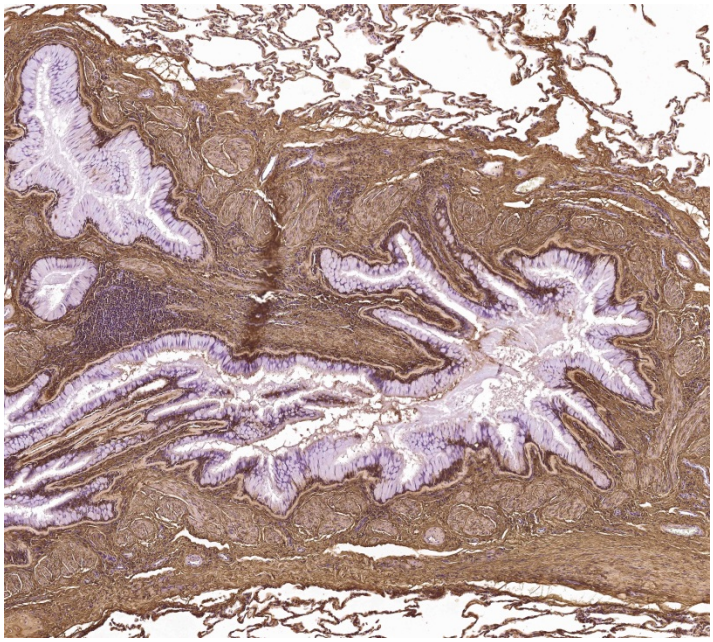
CLEVELAND CLINIC | Hyaluronan Matrices in Vascular Pathologies

HYALURONAN BINDING PROTEIN (HABP) DAB STAINING PROTOCOL

1.0 BACKGROUND

Although hyaluronan (HA) is non-immunogenic (i.e. antibodies cannot be raised against it) it can be detected in tissues and cells by use of a "hyaluronan – binding – protein" (HABP). Typically, HABP is isolated from bovine nasal cartilage and is comprised of either the G1 domain of aggrecan or a combination of G1 and the cartilage "Link" protein (both of which have conserved link-module HA binding domains). In the absence of the Link protein, G1 binding to HA is on/off. The combination of G1/Link, in the same preparation, stabilizes G1 binding to HA, causing it to remain bound to HA (Heinegard and Hascall (1974) *J Biol Chem* **249**:13, 4250-6). Biotinylated HABP permits the addition of a variety of fluorophore or enzymatic (i.e. horseradish peroxidase or alkaline phosphatase) streptavidin constructs for fluorescent or light microscopy. This protocol presents a histochemical approach for staining tissues and cells with HABP and the diaminobenzidine (DAB) substrate. A separate protocol is available for HABP fluorescent microscopy.



Slide Scanner Image (zoomed) of an asthmatic lung airway with accumulation of HA (Brown staining) with hematoxylin counterstain (purple staining).

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1.1 REAGENTS

Vectastain Elite ABC Kit: (Vector Labs PK-6101 Rabbit IgG. Store at 4 °C)

Goat Serum Blocker: 3 drops/10ml 1x PBS (or 150µl/10ml).

ABC Reagent: Add 2 drops of reagent A and 2 drops of reagent B to 10ml 1x PBS.

Additional Blockers: Avidin/Biotin Blocking Kit Vector Labs SP-2001. Store at 4 °C.

Biotinylated Hyaluronan Binding Protein (HABP): (EMD/Millipore/Calbiochem #385911, 50 ug). Add 100 µl water to 50 µg HABP. Vortex, and let sit at room temperature for 20 minutes. Vortex again, centrifuge and make 10 aliquots of 10 µl each. Store at –80° C indefinitely. Use at 1:100

ImmPACT DAB Peroxidase Substrate Kit: (Vector Labs SK-4105. Store at 4° C) Add 1 drop of chromogen to 1ml of diluent.

Clarifying Reagent: 1% HCl/75% Ethanol

Other Reagents: Hematoxylin (Fisher #22-220-102), Bluing Reagent (Thermo Scientific #7301), Flex 100 (Thermo Scientific #8101), Flex 95 (Thermo Scientific #8201), Clear Rite 3 (Thermo Scientific #6901).

Mounting medium: Cytoseal XYL Thermo Scientific (# 8312-4)

1.2 PROTOCOL

1. **Deparaffinize** slides thoroughly in two changes of Clear Rite (3 minutes each), Flex 100 (1 minute), Flex 100 (2 minutes), Flex 95 (1 minute), Flex 95 (2 minutes), running water (1 minute). Rehydrate in 1x PBS for 10 minutes.

2. **Avidin/Biotin Blocking Step:** First with Avidin/Biotin Kit. Block 15 minutes with Avidin D solution followed by rinsing in PBS. Then block 15 minutes with Biotin solution 18, then rinsing in PBS.

3. **Goat Serum Blocking Step:** Incubate sections for 20 minutes with Diluted Normal Goat Blocking Serum. Use 3 drops of the concentrated Normal Goat Serum per 10ml 1x PBS (or 150µl/10ml).

4. **Apply HABP:** Dilute HABP 1:100 in Diluted normal goat blocking serum. Decant blocker from slide onto a paper towel, by tapping the slide to the paper towel, and add the HABP to the section in a sufficient quantity to cover it. Let sit at room temperature for 1 hour.

5. **First Wash:** Decant the antibody solution onto a paper towel and transfer the slides into PBS. Let sit a few seconds, remove the slides, discard the PBS, replace the PBS and replace the slides for a second wash. Repeat this procedure for a total of 4 washes.

6. **ABC Reagent:** Incubate sections for 30 minutes with Vectastain ABC Reagent. Add 2 drops of reagent A and 2 drops of reagent B to 5 ml PBS and mix well. *Note*: Make up to 30 minutes before use.

7. **Second Wash:** Wash the slide as in step 5 and keep slides in PBS.

8. **ImmPACT DAB Substrate:** Add 1 drop of chromogen to 1ml of diluent and take slides to microscope. Apply substrate to sections and observe under the microscope until ideal staining has occurred, then immediately place slide in distilled water to stop the reaction (keep track of time). *Note*: 1 minute has worked well for lung sections, and 2 minutes has worked well for liver sections. Once all slides are in distilled water, rinse in running water for 1 minute prior to counterstain.

9. **Counterstain** for 2 minutes in hematoxylin, then rinse in running water for 10 minutes or until water looks clear.

10. **Clarify:** As a clarifier, make a 1% HCl/75% ethanol solution. Solution is very strong so just dip slides in once and immediately take it back out and place into water, and rinse in running water for 1 minute. Solution can be saved and reused if still clear.

11. **Bluing Reagent:** Place slides in Bluing Reagent for 1 minute, then rinse in running water for one minute.

12. **Dehydrate:** Dehydrate samples in 2 changes of Flex 100 (1 minute each), and Clear Rite (2 minutes).

13. **Mounting:** Allow slides to air dry after Clear Rite step, then mount with Cytoseal XYL and keeps slides at room temperature.