

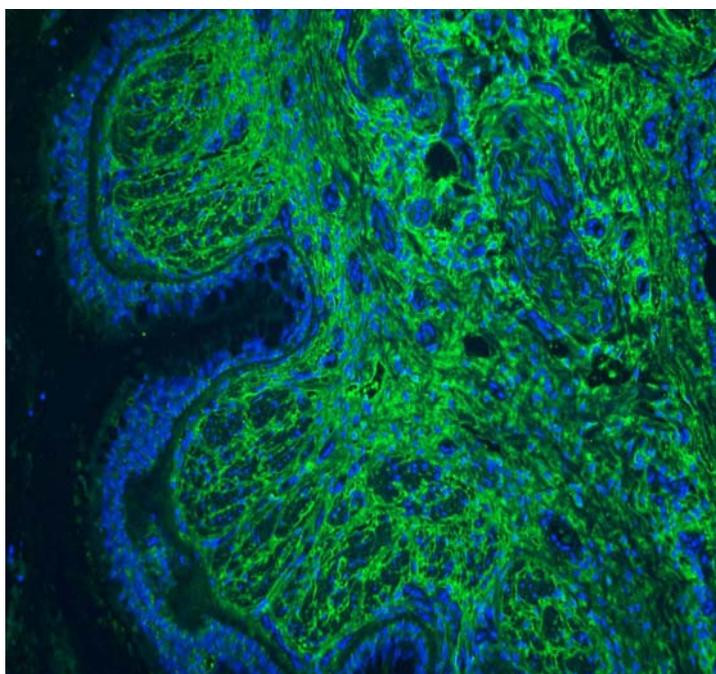


HYALURONAN BINDING PROTEIN (HABP) FLUORESCENT STAINING PROTOCOL

***PLEASE ACKNOWLEDGE NHLBI AWARD NUMBER PO1HL107147 WHEN YOU PUBLISH RESULTS USING THIS 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 method for probing tissues/cells with biotinylated HABP for fluorescent microscopy. A separate protocol is also available for a HABP histochemical approach for staining with the diaminobenzidine (DAB) substrate.



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Confocal image of a bronchus from a patient with asthma probed with HABP (green). Nuclei are shown in blue.

1.1 REAGENTS

Blocker (1% BSA in HBSS): Add 1 g bovine albumin (Sigma, A 2058) to 100 ml PBS (divalent cations are not necessary). Stir at room temperature for ½ hour until dissolved. Make aliquots of 10 ml each into ten 15 ml centrifuge tubes. Store at –20° C indefinitely.

Biotinylated Hyaluronan Binding Protein (HABP): (EMD/Millipore/Calbiochem #385911, 50 ug). Add 100 µl water to 50 mg 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

Fluorescent Streptavidin Construct: *Streptavidin, Alexa Fluor 488 conjugate (green)* (Molecular Probes, S-11223). Dissolve 1 mg in 1 ml PBS. Store at –4° C Use at 1:500

Fluorescent Mounting medium: VectaShield with DAPI (Vector Laboratories, H-1200)

1.2 PROTOCOL

1. Block: Once the tissue section is hydrated and equilibrated in PBS, place a suitable amount of blocker to cover it and let sit at room temperature for 30 minutes.

2. Apply HABP: Dilute HABP 1:100 (*mixed with another primary antibody in the same tube if you have one*) in blocker. Decant blocker from slide onto a paper towel, by tapping the slide to the paper towel, and add the HABP (*with second primary if you have one*) to the section in a sufficient quantity to cover it. Let sit at room temperature for 45 min.

3. First Wash: Decant the antibody solution onto a paper towel and transfer the slide to a slide jar containing 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.

4. Apply Fluorescent Streptavidin Construct: Dilute the fluorescent streptavidin construct 1:500 (*and any other secondary antibodies*) in blocker. Remove the slides from the jar and remove excess PBS surrounding the section with a KimWipe. Apply the streptavidin construct in sufficient quantity to cover the section. Incubate at room temperature for 1 hour in the dark.

5. Second Wash: Wash the slide as in step 4.

6. Mounting: To mount, remove a slide (*one at a time*), remove excess PBS surrounding the section with a KimWipe, and add a few drops of VectaShield to the section. Add a cover slip by placing one of the long ends on the slide and slowly lowering it like a sandwich. Repeat for remaining slides. To remove excess mounting media, place slides on a couple paper towels and put a couple more paper towels on top. Place a Styrofoam rack on top of the paper towels and add a small weight (*like an empty beaker*) to the top of the rack. Wait a minute or two and carefully disassemble the sandwich. Discard the paper towels and seal the slides by placing a generous amount of nail polish around the edges of the cover slip. Store at –20° C indefinitely.