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Immunohistochemistry for Hyaluronic Acid

- to detect the presence of hyaluronic acid in horseshoe crab cartilage

Day 1

1. Lay slides flat and incubate at 65˚C 15 min
2. Transfer slides to staining rack and clear in xylene 2 times 5 min
3. Rehydrate Slides
    a. Xylene 10 min
    b. 100% Ethanol 10 min
    c. 95% Ethanol 10 min
    d. 70% Ethanol 10 min
    e. Deionized water 10 min
4. Lay slides flat in a humidity chamber (slide box with wet paper towels on the bottom works fine)
5. Draw barrier around slides with hydrophobic Pap pen
6. Wash in PBS-Tween 20 Solution 10 min
7. Wash in .5% Triton-X solution 30 min
8. Rinse 2 times PBS-Tween
9. Wash PBS-Tween 3 times 5 min
10. Incubate in blocking solution 10 min
11. Incubate in primary antibody(sheep anti hyaluronic acid) in blocking solution overnight at 4˚C
    a. Concentrations used between 1:50 and 1:10,000

Day 2

1. Rinse 3 times PBS-Tween
2. Wash PBS-Tween 4 times 10 min each
3. Incubate in blocking solution 10 min
4. Incubate in 1:400 secondary antibody (donkey anti sheep) to blocking solution 30 min
    a. Keep slides in the dark from now on
5. Rinse PBS-Tween 2 times
6. Wash PBS-Tween 3 times 5 min
7. Incubate in 1:30,000 Hoechst 7 min
8. Rinse PBS-Tween 2 times
9. Wash PBS-Tween 3 times 5 min
10. Mount with Dako fluorescent medium

PBS-Tween20 Solution:
500 µL Tween 20
500 mL PBS

.5% Triton-X Solution:
250 µL Triton-X
50 mL PBS

Blocking Solution:
l mL goat serum
.2g BSA (bovine serum albumin)
9 mL PBS-Tween solution