

IHC for Ubiquitin/Aggregate Staining

Time: 2 days (5 hours day 1 and 4 hours day 2)

Materials

PBS

MeOH

H₂O₂-fresh

Normal Goat Serum (NGS)

Triton X-100

Avidin/Biotin Blocking Kit (Vector SP-2001)

1° antibody-rabbit anti-ubiquitin (DAKO z0458)

2° antibody-goat anti-rabbit-biotinylated (Vector BA-1000)

ABC Vectastain Elite Kit (Vector PK-6100)

DAB Substrate Kit (Vector SK-4100)

EtOH

Xylenes

Hematoxylin soln

0.3% ammonium hydroxide

Permount

<u>Blocking Solution</u>	<u>200 ml</u>
0.3% NGS	6 ml
0.3% Triton X-100	0.6 ml
0.3% H ₂ O ₂	2 ml of 30% H ₂ O ₂
in PBS	191.4 ml

Procedure

1A) From frozen tissue, thaw slides at RT until frost clears

1B) From paraffin embedded tissue, deparaffinize:

a-bake slides at 60°C for 30 min

b-xylenes 2x 5 min each

c-96% EtOH 2x 3 min each

d-90% EtOH 1x 1 min

e-80% EtOH 1x 1 min

f-diH₂O 1x 1 min

2) MeOH* + 0.3% H₂O₂ -20°C 10 min

*prechill MeOH soln

3) Wash 3x 10 min each with PBS

4) Block 1 hr RT in blocking soln + reagent A from blocking kit
(add 4 drops reagent A per 1 ml of blocking solution)

5) Wash 3x 10 min each with PBS

6) 1° antibody incubation 4°C O/N

Dilute antibody 1:1000 in blocking solution + 4 drops reagent B (from blocking kit) per 1 ml of blocking soln

7) Wash 3x 10 min each with PBS

- 8) 2° antibody incubation 1 hour RT
Dilute antibody 1:200 in blocking soln
- 9) Wash 3x 10 min each with PBS
- 10) ABC incubation* for 1 hr at RT
***Make ABC soln 30 min before use!**
per 5 ml PBS add:
2 drops reagent A
2 drops reagent B
- 11) Wash 3x 10 min each with PBS
- 12) DAB staining for 10 min at RT
DAB soln (from DAB kit)
per 5 ml diH₂O
2 drops buffer
2 drops DAB soln
2 drops H₂O₂
- 13) Rinse 3X with diH₂O
- 14) Counterstain and dehydrate with Hematoxylin
a-dip 10-15x in hematoxylin
b-rinse 4x with diH₂O
c-dip 5x in 0.3% ammonium hydroxide
d-rinse 4x with diH₂O
e-70% EtOH 1 min
f-85% EtOH 1 min
g-95% EtOH 1 min
h-xylenes 1 min
- 15) Mount with Permount mixed 1:1 with xylenes
let slides dry at least 1 hour to overnight