12-14-04 3B5H10 IFL on Human Tissues for Montese/Steve Finkbeiner
(paraffin embedded)
1:3000
1:1000
1:300
> 3 control cerebellums + 3 affected (blinded) = six slides
SF6 SF18
also 1:5000 CB Rb as a (+) control

1. 60°C to melt paraffin - 20 mins
2. deparaffinize 3x xylenes 5 min each
100% EtOH "
95% EtOH "
70% EtOH "
milli Q H_2O 2x 10 min ; [illegible]
3. Triton x-100 0.5% in PBS 2 x 15-20 min ; Tris wash - 5 min
4. Glycine Block - 0.3M in Tris 0.1M - 45-60 min
$\frac{75.07\,g}{mol} \times \frac{0.3\,mol}{L} \times \frac{L}{1000\,mL} \times 30\,mL = 0.67563\,g$ in 30 mL Tris 0.1M
5. rinse w/ PBS - 10 min, PBS-Tx - 10 min
6. block - NPS - 10% - 2.5 mL - 60 min - 120 min
25mls gel - 0.2% - 500 uL
milk - 2% - 500 mg
PBS-1x - 22 mL
0.5% triton in PBS tx 5mL
7. rinse PBS-1x 10 min
8. 1° Incubation O/N RT 1:300 3uL in 300uL
150 µL 1:1000 - 30uL (1:100) in 270uL
CB - 1:5000 1:3000 - 10uL (1:100) in 290uL
6 uL (1:100) for 300 uL
9. rinse - PBSTx 15 mm, PBS 15mm, PBS-Tx 15 m, PBS 15 min, PBSTx 15m
10. 2° incubation 1:300 cy2 α Ms (DK) - 3B5H10 - 10 uL
dilution: 1:300 cy3 α Rb (DK) - calbindin - 10 uL
3% NDS - 90 uL
gelatin - 60 uL
PBS 1x - 2.83 mL
11. rinses

Results: possibly too much autofluorescence
Glycine Block Not rigorous enough
Next: try 1% sodium borohydride / PBS
Proper controls: No 1° or 2°
No 1°
No Both 1° + 2°

SIGNATURE _____ DATE _____ 20__
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(A) 0.1M glycine - 0.3M in 1M Tris base 15-30 mins, wash w/ PBS

(A) If not fragile tissues
1% $NaBorohyd.$ in PBS for 30 min
wash w/ PBS: 1st 15 mins PBS
2nd 5 mins

10 mM citrate buffer pH 6.0 15min
90% formic acid 7 min
3 times
wash 1X PBS 10 min in each
3x 10 min

Serum ↔ dk goat | α Ms | α Rb