

Immunohistochemistry for BrdU/NEUN/GFAP fluorescence

Date

DAY 1

- 1) PBS washes 4 x 10 min each
- 2) 15 min 0.5% sodium borohydride in PBS
- 3) PBS washes 3 x 10 min each
- 4) 30 min 1 % triton X in PBS with 1 % H2O2
- 5) 10 min in DMSO (1%) in PBS
- 6) PBS washes 3 x 10 min each
- 7) 60 min 2N HCL (RT)
- 8) PBS washes 3 x 10 min each
- 9) 15 min 0.5% sodium borohydride in PBS
- 10) PBS washes 3 x 10 min each
- 11) 30 min in PBS albumin (10%) and Tx (0.3%)
- 12) Primary Antibody BrdU. Anti rat 1:800 (serotec) in PBS albumin (1 %) and TX (0.32%)
minimum 20 hrs.

DAY 2

- 13) PBS washes 3 x 15 min each with TX (0.02%) and albumin (1%)
 - 14) Biotinylated 2ndary antibody, anti rat IgG 1:500 in PBS, albumin (1%) and TX (0.32%)
3 hs
 - 15) PBS washes 3 x 10 min each with TX (0.02%)
- ***Half an hour before made up elite AB (2 drops of A, 2 drops of B in 10 ml PBS)
- 16) Sections in AB 90 min,
 - 17) PBS washes 3 x 10 min each

18) kit cumarina 1:100 **slides in the dark**

19) PBS washes 2 x 10 min each

20) Primary Antibody NeuN mouse 1:250 in PBS albumin (1 %) and TX (0.32%)
minimum 20 hrs.

DAY 3

21) PBS washes 3 x 15 min each with TX (0.02%) and albumin (1%)

22) Biotinylated 2ndary antibody, anti mouse IgG 1:300 in PBS, albumin (1%) and TX (0.32%)
3 hs

23) PBS washes 3 x 10 min each with TX (0.02%)

***Half an hour before made up elite AB (2 drops of A, 2 drops of B in 10 ml PBS)

24) Sections in AB 90 min,

25) PBS washes 3 x 10 min each

26) kit CY3 1:100 **slides in the dark**

27) PBS washes 2 x 10 min each

28) Primary Antibody GFAP rabbit 1:500 in PBS albumin (1 %) and TX (0.32%)
minimum 20 hrs.

DAY 4

29) PBS washes 3 x 15 min each with TX (0.02%) and albumin (1%)

30) 2ndary antibody, anti rabbit Alexa 1:250 green in PBS, albumin (1%) and TX (0.32%)
3 hs

31) PBS washes 3 x 10 min each with TX (0.02%)

32) PBS washes 2 x 10 min each