

## **Immunohistochemistry for BrdU**

### Date

- 1) TBS washes 5 x 3 min each
  - 2) 30 min 1 % triton X in TBS with 1 % H<sub>2</sub>O<sub>2</sub>, shaker cold.
  - 3) TBS washes 4 x 3 min each
  - 4) 60 min 2N HCL (RT)
  - 5) TBS washes 2 x 3 min each
  - 6) 15 min 0.5% sodium borohydride (1g/200 ml TBS)
  - 7) TBS washes 4 x 3 min each
  - 8) 30 min in TBS albumin (10%) and Tx (0.3%)
  - 9) Primary Antibody BrdU. Anti mouse 1:2,000 in TBS albumin (1 %) and TX (0.32%)  
on shaker cold, minimum 16 hrs.
  - 10) TBS washes 4 x 3 min each with TX (0.02%) and albumin (1%)
  - 11) Biotinylated 2ndary antibody 1:500 in TBS, albumin (1%) and TX (0.32%)  
2 hrs shaker, RT
  - 12) TBS washes 4 x 3 min each with TX (0.02%)
- \*\*\*Half an hour before made up elite ABC (2 drops of A, 2 drops of B in 10 ml)
- 13) Sections in ABC 90 min, shaker RT
  - 14) TBS only washes 4 x 3 min each
  - 15) DAB as indicated in the kit or according to the solution below
  - 16) TBS washes 3 x 3 min each

DAB solution for 50 ml, 45 ml of deionized water, 30 mg of DAB 36 microliters of H<sub>2</sub>O<sub>2</sub> (30%) add 5 milliliters of nickel solution (1%).