

## **BRDU, NST Immunocytochemistry**

**Block solution:** 2 mg BSA / mL PBS  
4% normal donkey serum  
0.05% Triton-X

### ***Day 1***

- Wash 2 x 10 min with PBS
- Treat with 2N HCl, 0.1% Triton for 30 min at 37°C
- Treat with 0.1M Na Borate pH 8.5 for 30 min at 37°C
- Wash 2 x 10 min with PBS
- Incubate with block solution for 1 hour at room temperature
- Incubate with mouse anti-BrdU at 1/4000 in block solution at 4°C overnight

### ***Day 2***

- Wash 2 x 10 min with PBS
- Incubate with unconjugated Fab goat-anti-mouse at 1/500 in 0.05% NDS for 1 hour at room temperature
- Wash 2 x 10 min with PBS
- Incubate with Cy3 donkey anti-goat at 1/500 in 0.05% NDS for 1 hour at room temperature
- Wash 2 x 10 min with PBS
- Incubate with NST at 1/500 in block solution overnight at 4°C

### ***Day 3***

- Wash 2 x 10 min with PBS
- Incubate with FITC donkey anti-mouse at 1/50 in 0.05% NDS for 1 hour at room temperature
- Wash 2 x 10 min with PBS
- Coverslip with Mowiol