

# **BrdU staining protocol (frozen sections)**

(prepared by P. Sun)

## **Materials:**

BrdU (Sigma Cat#B5002)

Anti-BrdU antibody(Rat monoclonal) (abcam Cat#ab6326)

Alexa Fluor® 488 donkey anti-rat IgG (H+L) (invitrogen, Cat#A-21208)

DAPI (invitrogen, Cat#3571)

Mounting medium (Vector, Cat#H-1000)

## 1. BrdU injection

- (1) Dissolve BrdU (10 mg/ml) in sterilized PBS. Aliquot and freeze down at -20°C until needed
- (2) Thaw BrdU at RT. Invert tube until the solutions are clear
- (3) Inject mouse (intra-peritoneal) with 10 µL/gram of body weight of BrDu solution (10 mg/ml).
- (4) Wait for about 2 hrs for cell proliferation.

## 2. Dissection of tissue

- (1) Sacrifice the mouse, dissect tissue.
- (2) Embed tissue in OCT and immediately freeze on dry ice.
- (3) Store tissue at -80°C until section.

## 3. Immunostaining

- (1) Circle sections (6-8 um) with PAP PEN
- (2) Fix sections in 4% formaldehyde for 30 min at RT.
- (3) Wash 3 x 5 min in 1 x PBS at RT.
- (4) Incubate in prewarmed (37°C) 1N HCL for 45 min-1 hr in 37°C incubator in the lab.
- (5) Wash 3 x 5 min in 1 x PBS at RT.
- (6) Blocking non-specific surfaces with blocking buffer for 30 min at RT
  - Blocking buffer A (for regular staining): 20% NGS, 0.1% tritonX-100, 0.02% NaN<sub>3</sub>, 0.05% Tween-20, 1 x PBS
  - Blocking buffer B (If co-staining with another primary antibody that is anti-GOAT): 5% BSA, 0.1% tritonX-100, 1 x PBS
- (7) Add BrDU antibody (1:500 in dilution buffer) and incubate in a humidified box for ON at 4°C
  - Dilution Buffer A (for regular staining): 2% NGS, 0.1% tritonX-100, 0.02% NaN<sub>3</sub>, 0.05% Tween-20, 1 x PBS
  - Dilution Buffer B (If co-staining with another primary antibody that is anti-GOAT): 0.5% BSA, 0.1% tritonX-100, 1 x PBS
- (8) Wash 3 x 5 min in 1 x PBS at RT.
- (9) Add secondary antibody, FITC-donkey anti-Rat IgG (1:500 in dilution buffer as shown above) and incubate for 1 hr at RT
- (10) Wash 3 x 5 min in 1 x PBS at RT.

- (11) DAPI stain: add 0.1  $\mu\text{g/ml}$  DAPI in 1X PBS and incubate for 10-15 min at RT
- (12) Wash 3 x 5 min in 1 x PBS at RT.
- (13) Mount with mounting medium and seal coverslip.