BrdU Injections & Immunocytochemistry

Objective: To label newly generated cells in the adult mouse brain

- BrdU injection:

Adult mice (6-10 weeks old used in this study) are injected every other day for a total of 3 times (50 mg/kg, **i.p.**)

BrdU source (lypholized powder from Upstate Cat#19-160, 4X25mg packing). (this is much more soluble than the Sigma product, which you have to weigh to make solution-not that easy)

- Adult mice are sacrificed at 24 hrs after the last injection through intracardial perfusion of 4% PFA (in 0.1M PBS, pre-cleared with cold 0.1M PBS). Brains are dissected out, post-fixed in PFA overnight. Protected in 30% sucrose (made in dd water).

- Cryosectioning of mouse brain into 40 um. Collect the sections in ice-cold 0.01M PBS, rinse on ice in PBS for 30min.

- Denaturing DNA:

Transfer the sections to 2N HCl (water solution) in a beaker, seal with Parafilm. Keep in RT for 30min (treatment time is very important; do not prolong the treatment)

- Transfer sections to borate buffer (pH=8.5), keep in BF for 20 min to neutralize slice pH conditions.

- Transfer sections back to 0.01M PBS, briefly rinse 3X5 min.

- Primary antibody incubation:

Antibody source: I found that rat anti-BrdU (from Accurate Chemicals) is the best for IHC results

Incubate secitons in rat anti-BrdU (1:1000 diluted in PBS) in small volume (~500 ul) overnight.
Rinse with PBS 3X5 min.

- Secondary antibody application:

This is typically done with multi-fluorophore labeling. So plan carefully. An Alexa-633 conjugated donkey anti-rat antibody produces very good results (see the sample labeling below)

Incubate the section in secondary antibody cocktails for overnight in 0.01M PBS.

Rinse section for 20min in 0.01M PBS.

- Mount sections with Vectashield anti-fading medium.