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We use this protocol and it's working

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EdU Immunohistochemistry using Click-it reaction

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ABSTRACT

This protocol allows users to image histology tissue using basic immunohistochemistry techniques in combination with EdU capture (using the Click-it reaction system). EdU (5-ethynyl-2'-deoxyuridine) will capture S-phase cells with the thymidine analogue, allowing to take a snap-shot of actively diving cells. In this assay the modified thymidine analogue EdU is efficiently incorporated into newly synthesized DNA and fluorescently labeled with a bright, photostable Alexa Fluor™ dye in a fast, highly-specific click reaction. This fluorescent labeling of proliferating cells is accurate and compatible with antibody methods due to the mild click protocol.

MATERIALS

1. dU 5-ethynyl-2'-deoxyuridine (200mg/kg/mouse)
2. BD Fix/Perm Solution (cat #: 554714)
3. 30% Sucrose Solution
4. Tissue-Tek O.C.T. Compound (cat#: 4583; Sakura)
5. Tissue-Tek Base Mold System (Sakura): size will depend on organ of interest
6. SuperFrost Plus slides (cat #: 12-550-15; Fisher)
7. Click-iT™ EdU Cell Proliferation Kit for Imaging, Alexa Fluor™ 555 dye (cat #: C10338; Thermo): A488, A555, A594, A647 work very well!
8. Optional: TruStain FcX™ (anti-mouse CD16/32) Antibody (cat#: 101319; Biolegend)
9. Optional: primary/secondary antibody needed for wanted panel
10. Optional DAPI
11. Optional Flouromount G (cat #: 00-4958-02, Thermo)

Tissue Preparation and Sectioning

1d 4h 30m

1 Inject animals with EdU as outlined in animal protocol license (typically by performing an intra-peritoneal injection with 200 mg/kg per mouse). 30m

1.1 Euthanize animal and harvest organ of interest: place tissue in 0.1% BD cytofix/cytoperm 4h

fixative (0/N at 4C).
Time of euthanasia after EdU injection will vary widely on organ/cell of interest, and age of the animal. For an optimal time window- prior literature should be investigated. For fast proliferating tissues (such as intestinal epithelium or skin), a good starting point will be around 1-4hrs; for developing embryos, 20-30min might be sufficient.

1.2 The following day, place fixed in tissue in 30% Sucrose Solution (allowing the tissue to get dehydrated). 1d

1.3 Embed tissue in Tissue-Tek Base Mold System by orienting the tissue in its optimal configuration in OCT Mounting Media. Freeze tissue on dry ice until OCT turns from a viscous clear compound to a solid white. OCT blocks can be stored at -80C long term (months). 1h

1.4 Section OCT blocks on a cryostat at desired thickness on SuperFrost Plus slides. Tested so far are up to 50um in thickness. If desired, sectioned slides can be stored at -20C long term (months). 2h

Tissue staining with EdU, antibodies, and Mounting 1h 5m

2 Place tissue slides in humidity chamber, and hydrate tissue sample with PBS. 5m

2.1 Block tissue section using Blocking Buffer (composed with 0.3% Triton, Fc-block, and 1x BD Perm/Wash (+ Fc-block if needed)). 1h

2.2 Stain sample with EdU Click-it reaction solution following the instructions from the manufacturer. EdU staining is extremely bright- 30min will be sufficient to stain efficiently a 25um section. 45min staining is advised for thicker sections. The copper concentrations typically used in traditional click chemistry reactions can affect fluorophores such as GFP, mCherry, R-PE, and R-PE tandem dyes. Highly recommend to 1) stain for EdU before other antibodies (either conjugated-primaries or secondaries), and 2) stain with anti-fluorophore antibodies (such as anti-GFP). 30m

2.3 Wash the samples 3 x 5min with PBS 15m

2.4 Continue staining samples with wanted panel (primary, secondary and/or Nuclear Dye (such as DAPI)). For follow-up quantification (for example: % EdU+ cells), DAPI staining is very much important. 1d

2.5 Mount samples with preferred mounting media (recommended is Fluoromount G). 30m