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EdU Click-iT Administration

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We use this protocol and it's working

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Abstract

This protocol uses the Thermo Fisher Click-iT EdU Imaging Kit (I used the 647 fluorophore, C10640, https://www.thermofisher.com/order/catalog/product/C10640).

Materials

Thermo Fisher Click-iT EdU Imaging Kit

Prepare EdU Stock Solution

1 Add 2 ml of DPBS to 5 mg EdU stock vial (component A).

2 Aliquot into 100 µl aliquots and store at -80°C.

3 This makes a 10 µM solution of EdU (2.5 µg/µl).

EdU Injection into Pups - IP Injection

4 Must inject 10-15 µg of EdU/gram of animal weight, so 4-6 µl of working 10 µM stock per gram of animal weight.

5 Thaw out appropriate number of aliquots depending on weight and number of pups/mice you wish to inject. Combine into one vial and take to the mouse house. For CD1/bl6 pups, P3 pups weigh ~3 g for example.

6 Pull all volume into syringe to inject pups as you weigh them. I inject 6 µl/g with a 2 ml syringe (this syringe uses ~70 µl of void volume, so add that to your total volume).

7 Weigh one pup at a time and inject IP appropriate volume (I round up for injection, i.e., 3 g pup would get 18 µl of 10 µM stock, so 20 µl injection).

Tissue Collection and Staining

8 Collect tissue as normal, section, block with NGS.

9 After blocking, follow Click-iT EdU Thermo Fisher staining protocol (Starting with “Detect EdU section, step 4.1).

10 EdU staining takes ~1 hr.

11 After EdU staining, can continue with regular primary antibody incubation/staining protocol as needed.