EdU labeling protocol (by Connor McGuire and Matthieu Paiola, May 3, 2024)

1- Thaw EdU 10ug/ul aliquot at 50 °C for few minutes and vortex until the little specks of powder are dissolved
2- Inject frog with 2.5ul of EdU 10ug/ul per g of bodyweight or 1ul of EdU 10ug/ul or exposed cells to 10ng/ul EdU.
3- After 24h/18h proceed for cell isolation.
4- Perform extracellular staining on ice (2B1 sup' for 1h, 1/300 goat-anti-mouse IgG-FITC for 30 min, 1/300 biotinylated AM22 for 30 min, 1/100 PE-streptavidin for 15 min, wash with aPBS, after spin poor out the sup' and add 1ul of Ghost Dye UV 450 (Tonbo bipsciences) for 30min, wash with staining buffer (1% BSA and .05% sodium azide)
5- After wash (spin at 13,000g for 1min at 4°C, and sup' poured out) resuspend by vortexing (two brief bursts) or pipetting up and down if the pellet is sticky
6- To about 100ul of cells add 50ul of 4% PFA (aliquots are kept at -20°C), flick the tubes and incubate for 10 min on ice (The timing is important, a too long fixation will impact the EdU labeling)
7- Add 1 mL of staining buffer and spin at 2,500 rpm (700g) for 5 min at 4°C. The sup is then poured out. The experiment can be stopped here to resume the next day (Keep the samples at 4°C protected from light).
8- Vortex briefly and permeabilize by adding to about 100ul of cells 50ul of permeabilization buffer (0.5 % Triton-100X in aPBS), flick the tube very gently once or twice and incubate on ice for 30 min.
9- Wash: add 1ml of staining buffer and spin at 2,500rpm (700g) for 5min at 4°C. The sup' is poured out
10- Add 500ul of reaction cocktail (440 ul of Tris-HCl 100 mM pH 7.6, 10ul of CuSO4 100 mM, 50ul of Sodium Ascorbate 0.02g/mL, 1uL of Cy5 Sulfo-Azide Dye). Be sure to add Sodium Ascorbate last, do not prepare more than 5 min in advance.
11- Incubate 30 at RT protected from light
12- Wash with 1mL
13- Wash with 1mL
14- Ready for flow measurement

Solution:

EdU (A10044, Invitrogen, Waltham MA, USA): powder dissolved at 10ug/ul (40 uM) in aPBS, heat briefly at 50°C, aliquots are kept at -20°C
CuSO4 is dissolved at 100 mM in distilled water
Tris-HCl= 1M stock solution at pH 8 (Invitrogen) diluted by 10 and adjusted to pH 7.6 with NaOH and keep at 4°C
Sodium Ascorbate= dissolve powder at 0.2g/mL (do not handle powder form with metal), keep aliquots at -20°C
Cy5 Sulfo-Azide Dye (Catalog: A3330, Lumiprobe, Hunt Valley, MD USA) resuspended in 750ul of DMSO and keep aliquot at -20°C

Notes:

Ghost Dye UV 450 can be detected with the violet laser at 450