**EdU Click-iT labelling protocol. Rosello-Diez lab**

Our lab has been using this approach to develop EdU in cells pulsed with 10 uM EdU (1-1.5h) and in tissue from either mice injected i.p. with 25-50 mg/kg EdU or mice that received EdU in drinking water at 200 ug/ml for 1 week. We use Edu from Carbosynth or Santa Cruz (sc-284628) as it is considerably cheaper than from other vendors.

**Stock solutions:**

EdU: 20 mM in PBS (50 mg in 10 ml). Filter-sterilse!

Sulfo-FAM-Azide: 4 mM in H2O (3 mg in 1 ml H2O) (2-5 uM in final reaction, Lumiprobe #A1330, or D1330 for Cy3)

CuSO4: 100 mM in H2O (in final reaction 4 mM)

Ascorbic acid 200 mg/ml (FRESH!!) (in final reaction 20 mg/ml)

Make label mix just before use by combining (in this order; precipitate is formed after addition of CuSO4 to PBS, this is dissolved after addition of ascorbate):

PBS 860 µl

CuSO4 40 µl

Azide-dye 0.5 µl

Ascorbic acid 100 µl

1. Wash OCT and rinse tissues 2 x 5min in PBS

2. Permeabilize cells/tissue for 10-30min in PBS + 0.5% Triton X-100 (depends on thickness)

3. Rinse tissues 2 x 5min in PBS

4. Incubate 15-30min in EdU development cocktail made immediately prior.

5. Rinse tissues 2 x 5min in PBS

Other labs tested sulfonated vs. non-sulfonated fluorophore-azides and found that the sulfonated versions are necessary to avoid general tissue staining in aqueous buffers. Lumiprobe currently has sulfo-Cy3/5/7 azides available. We reconstitute these azides in water to create 2 mM stocks. These can be used in aqueous buffers without organic solvent and don't require any special destaining.