EdU detection in Formalin-fixed paraffin sections with ClickiT AlexaFluor 488 kit

1. **Deparaffinize:**

Xylene	2 X 5 min	**Prepare**
100% EtOH	2 X 3 min	PBS+3%BSA
95% EtOH	2 X 3 min	
70% EtOH	3 min	

2. Rinse with water to remove EtOH.

3. Draw circle with pap pen

4. Block for 10 minutes with PBS+3% BSA

5. Prepare **Click-iT reaction cocktail** (need approx 100 µl/section);
   use within 15 minutes of preparing it.
6. Per **1 ml**:

(1) **860 µl 1x Click iT reaction buffer (Component D)**
   (diluted fresh with dH2O from 10x reaction buffer concentrate "Component D")
(2) **40 µl component E** (CuSo4)
(3) **5 µL AlexaFluor 488 azide** (one that comes with kit; made by mixing 70 µl
   component C (DMSO) with component B until dissolved)
   (A10266; purchased separately MW = 861.04;
   0.5 mg dissolved in 100 µl DMSO=5.81 mM stock; , store at -20 for up to 1 yr)
   2 µl per 1 ml cocktail=11.6 µM final)
(4) **100 µl Reaction buffer additive (Component F)** (diluted fresh from
   10x stock with dH2O;
   10x stock is made by adding 2 ml dH2O to Component F vial,
   mixing until dissolved; store at -20 for up to 1 yr.
   Once 10x stock is diluted, reaction buffer additive must be used that day)

7. Add **Click-iT reaction cocktail** to sections; incubate for 30 minutes at room temp,
   protected from light

8. Wash 1x for 5 min with PBS+3%BSA

9. Wash 1x for 5 min with PBS

10. Dilute Hoescht 33342 (**Component G)** 1:2000 in PBS, apply to sections for 30 min,
    protected from light. Remove Hoescht

11. Thaw ProLong Gold (No DAPI)

12. Wash 3x for 5 min with PBS

13. Mount with ProLong Gold (no dapi), cure in dark for 24h
    Use this instead of permount