

## **Fluorescent in situ protocol using TSA Plus Fluorescence Systems**

Follow normal in situ hybridization protocol through DAY2 antibody blocking step.

Incubate with 1:1000 anti-Dig POD antibody (150U/ul) overnight 4°C.

### **DAY 3**

1. Wash in MAB 5x 60 min at RT (can do one ON @ 4°C).
2. Wash 5 min with 1X Plus Amplification Diluent.  
Prepare Fluorophore Tyramide Amplification Reagent working solution:  
"Thaw" 50X DMSO- Fluorophore tyramide stock solution.  
Dilute 1:50 with 1X Plus Amplification Diluent.
3. Incubate sample for 10min Tyramide working solution.
4. Rinse 4X 15 min and 2X 30min with PTw.
5. Fix 1 hr in MEM-FA.
6. Dehydrate in Methanol or isopropanol.
7. Clear with BBBA.

### **For GFP counterstain:**

Add 1:500 anti-GFP with 1:1000 anti-dig POD in overnight incubation.  
After 10 min tyramide incubation do 3X5min washes.  
Add fluorescent anti-rabbit 2° for 2-4hrs at RT. Wash at least 4X30 min.