Planarian antibody staining

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1. Kill up to 15 worms in 5% N-acetyl cysteine (NAC) in 1xPBS for 5-10' with rocking in eppendorf tubes
   Note: NAC goes bad over a few months. Thus the time in NAC is constantly changing: 5min for fresh, 10min for 1+ months old.
2. Quickly pipet off as much NAC as possible
3. Pipette on Carnoys fix (6:3:1 EtOH:Chloroform:Acetic acid) and immediately invert tube and keep animals swirling to avoid clumping until worms are equilibrated
4. Fix for 2hrs at 4°C with rocking
5. Rinse 1x with MeOH (can now store worms in 100% MeOH for several weeks at -20°C)
6. Rinse 1x 5min with 1:1 MeOH:PBSTx
7. Wash 2x in PBSTx
8. Bleach O/N at RT NO rocking in 3% formamide, 6% H_2O_2, in PBSTx
9. Block 2hr. in PBSTx**S** (10% Horse Serum) at RT
10. Incubate in 1° antibody in PBSTxS O/N at RT with rocking in 24-well plate. For ~10 animals you can use ~300µl (for anti-H3P use at 1:500).
11. Wash 2x quickly in PBSTx (save antibody!), and then wash 6x 1ml over the next 2 hrs in PBSTx with rocking.
12. Incubate in 2° antibody in PBSTxS O/N at RT with rocking (currently using Zymed-HRP and Jackson antibodies at 1:200). 300µl per well.
13. Wash the same as in step 11.
14. Put animals in 500µl of PBSTx and add Cy3-tyramide @ 1:500 (1µl)
15. Add 1:2000-1:10000 freshly diluted H_2O_2(30%) in water to the tyramide mix to get reaction started.
16. Develop for 5-10min
17. Wash 3x quickly in PBSTx then look on scope. If stain is good, you can clear right away. If background is high, wash O/N and check stains the next day.
18. Clear @ 4°C in ~500µl of 80% glycerol + few drops of vectashield.

Solutions

10x PBS

NaCl	80g
KCl	2g
Na_2HPO4	14.4g
KH_2PO4	2.4g
H_2O	To 800mL

pH to 7.4

Then, bring to 1L with H_2O (filter)

PBSTx

1XPBS

0.3-0.5% TritonX-100

PBSTxS

1XPBSTx

5-10% Horse Serum