Antibody Staining Protocol with TSA amplification

1) Wash embryos 2x 30 minutes in PBTw with rocking.
2) Block for 30 minutes in 100 µL PBTw + 5% NGS with rocking.
3) Add the primary antibody (at the recommended dilution in PBTw +NGS). Make sure the embryos are properly mixed.
4) Incubate overnight at 4°C.
5) Recover the primary antibody to use again by transferring to another tube.
6) Wash embryos 3x quickly (let embryos sink, then remove liquid) with PBTw.
7) Wash 3x 30 minutes with PBTw with rocking.
8) Block 30 minutes in 100 µL PBTw + 5% NGS with rocking.
9) Add biotinylated secondary antibody (at the recommended dilution in PBTw +NGS, usually 1:300).
10) Incubate at room temperature for 60 minutes (no rocking necessary).
11) Wash 3x quick with PBTw.
12) Wash 3x 30 minutes with PBTw.
13) Block for 30 minutes in 100 µL PBTw + 5% NGS with rocking.
14) Incubate 60 minutes in streptavidin-HRP (Jackson Labs 1:100 dilution).
15) Wash 3x quick with PTw.
16) Wash 3x 30 minutes with PBTw. BE SURE TO REMOVE ALL PTw AFTER LAST WASH.
17) Incubate 15-20 minutes in TSA reagent (TSA-Cy3 or TSA-Cy5 diluted 1:50 in TSA amplification diluent).
18) Wash 3x quick with PBTw.
19) Wash 3x 10 minutes with PBTw.
20) Embryos are ready for the next step (ie mounting or the next antibody).

Solutions:

PBTw:
1X PBS
0.1% Tween-20
0.1% BSA (bovine serum albumin)

PBTw + NGS:
1X PBS
0.1% Tween-20
0.1% BSA (bovine serum albumin)
5.0% NGS (normal goat serum)

TSA kits (Perkin Elmer)
NEL-705A – TSA Cy5 kit
NEL-704A – TSA Cy3 kit
NEL-701 – TSA FITC kit