Antibody Staining Protocol

Note: All washes are done with 1ml of PBtw with rocking

1) Rehydrate embryos by removing methanol and adding PBtw
2) Wash 2X 30 minutes in PBtw
3) Incubate 30 minutes in 100 µL PBtw + NGS
4) Add the primary antibody. The antibody should be diluted in PBtw + NGS
5) Let sit overnight at 4°C, no rocking necessary
6) Wash 3X quick with PBtw
7) Wash 3X 30 minutes with PBtw
8) Incubate 30 minutes in 100 µL PBtw + NGS
9) Incubate 2 hours to overnight at 4°C in secondary antibody diluted in PBtw + NGS, no rocking necessary
10) Wash 3X quick with PBtw
11) Wash 3X 30 minutes with PBtw
12) Embryos are ready to mount in 70% glycerol or may be stored at 4°C

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)