

## 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  $\mu$ L 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  $\mu$ L 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)