ABC ANTIBODY LABELING

Source: Zoltan Varga; this protocol is a derivative of a protocol kindly provided by Cecilia Moens)

1. FIX 2-3 h at RT in 4% PFA

2. Rinse 3x 5 min in PBST (use PBS, if you intend to use ProK)

3. PERMEABILIZATION (in addition to Tween, 2 more optional alternatives to try)

Tween	**Acetone**	**ProK**
No additional permeabilization steps necessary	Rinse 1x PBST	10 µg/ml in ddH_20
Leave in PBST	1x ddH_20 5 min at RT	Rinse 3x in ddH_20 (rapid!)
Continue with BLOCK	Acetone 7 min –20°C	Rinse 3x 5min in PBS
	1x ddH_20 5 min at RT	Fix in PFA 20 min at RT
	1x PBST at RT	Rinse 3x 5min in PBS

4. BLOCK (2% Sheep serum,1% BSA, in PBS (if ProK was used) or in PBST)
1h at RT

5. PRIMARY ANTIBODY (Mouse IgG)
diluted in Blocking solution
dilute according to manufacturer's recommendations
overnight at 4°C on shaker

6. Wash 6x 15 min in PBS or PBST

7. SECONDARY ANTIBODY (anti Mouse IgG-Biotinylated)
1:500 in Block
2-4h at RT

8. Wash 6x 15 min in PBS or PBST

9. ABC REACTION
10µl A Solution
10 µl B Solution
1 ml Block
Mix A and B solution in BLOCK (PBS or PBST), 30 min ahead of incubation (during quenching and washing steps)

10. QUENCH

Incubate embryos in 5% H_20_2 in absolute Methanol
30 min at RT

11. wash 3x 5 min in BLOCK

12. ABC INCUBATION

Incubate ABC mix and embryos for 2h at RT

13. Wash 4x 15 min in PBST

14. DIAMINOBENZIDINE (DAB) REACTION

1 mg DAB
1 ml PB pH 7.3
1 ml ddH_20
20 µl DMSO

16. presoak Samples for 15 min in DAB solution

17. after presoak add 5 µl 3% H_20_2 to unused solution
discard presoak
incubate in H_20_2/DAB
watch reaction

18. stop with ddH_20, rinse several times
19. rinse in PBST
20. mount tissue in 2,5% - 4% Methyl cellulose or dehydrate for resin mount.