Xenopus Double In-Situ

Perform in-situs as for single in-situ, add both fluorescein and digoxigenin labeled probes to hyb mix on day one.

Probe	Label With	Color Rxn With
Stronger	fluorescein	BCIP/NBT, develop first, can develop at 37°C
Weaker	digoxigenin	Fast Red (can also try Magenta Phos) 37°C OK

Use BCIP/NBT for the first chromogenic reaction as Fast Red is not color fast in alcohol.

DAY 3

Following first chromogenic reaction stop the reaction with the following washes:

-3 x 5' in 1X MAB
-10' MAB + 10mM EDTA
-5' each 75% MAB/ 25% Methanol
50% MAB/ 50% Methanol
25% dH2O/ 75% Methanol
100% Methanol
Rehydrate into MAB through solutions as above

-Re-block for 1-2hrs in MAB + 2% BMB + 20% HIGS
-Antibody incubation 4hrs RT or 4°C overnight. Use 1:2000 dilution anti-Dig AP or 1:4000 dilution anti-Fluorescein AP

DAY 4

-Wash in MAB: 5 x 60 min at RT. (one can be O/N at 4°C if Ab incubation 4 hrs at RT)
-Wash embryos 2 x 5 minutes in 0.1M Tris pH8.2
-Incubate in Fast Red until red staining is evident (Roche 1496549)
(Dissolve 1 Fast Red tablet in 2ml 0.1M Tris pH8.2, filter to eliminate any undissolved Fast Red).
-Post-fix embryos in MEMFA.
-Wash embryos in PBTriton (0.1%) or PTW following reaction.
-Store at or 4°C in above solutions or at -20°C in 50% glycerol (made up in 1X PBS).