

Generic Whole Mount Antibody Staining Protocol

C. Moens, June 2004

- Fix embryos in 4%PFA/PBS, overnight at 4°C.
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- Wash 4X 5minutes each in PBST.
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- Permeabilize with 10ng/ul proteinase K in PBST as for RNA in situs
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- Stop protK with 4%PFA/PBS for 20 minutes¹
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- Wash 4X 5minutes each in PBST.
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- Block in PBST + 2% Goat Serum + 2mg/ml BSA + 0.1% Triton, 1 hour at RT.
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- Add 1° antibody to same solution and incubate overnight at 4°C.
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- Wash 5 times, 30 minutes each in PBST + 1% DMSO + 0.1% Triton (PBSDTT).
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- Re-block in PBSDTT + 1% sheep serum for 1 hour at RT.
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- Add 2° antibody to above (1:100 Alexa-Fluor conjugated), incubate O/N at 4°C.
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- Wash several times in PBSDTT.
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- For flat mounting, dehydrate through 25%, 50% and finally 70% glycerol in PBST and mount in glycerol or anti-fade reagent.

¹ note: I am not sure it is necessary to re-fix at this point. Probably sufficient to wash quickly in PBST and then proceed into block, which will sop up any remaining ProK.