made by Masahiro Shin (12/31/2019)
(Masahiro.Shin@umassmed.edu)

Whole mount immunostaining of zebrafish embryos

- this protocol is effective on embryos up to 4 dpf
- note that deeper tissue will be much harder to stain after 32 hpf
- this protocol generally works for most antibodies we have tried. For goat polyclonal antibodies (e.g. those zebrafish Abs from R&D) use the alternative protocol on our website.

Fix with 8%PFA/PBS 4°C for O/N
Wash with 100%MetOH once
Quench embryos with 3%H_2O_2 in MetOH for 1hr
Wash with 100%MetOH twice
Keep embryos until use

<1st day>
Rinse embryos with PBSTw (PBS+0.1%Tween20) a few times
Rinse with water once
Permeabilize embryos with water for 30min at RT (up to 38hpf)
Permeabilize embryos with PBSTw/1% TritonX100 for 30min~1hr
Rinse with water once
Rinse and incubate embryos with Citrate buffer (0.1M tri-sodium Citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$, MW=294.1, #S1804-SIGMA)/0.05% Tween20 pH6.0 with HCl) for 15min at RT
Heat treatment of embryos in Citrate buffer **at 94-98 °C for 20min** (mix embryos by tapping tube every 5min x4!) on heat block.
Let cool to room temperature.
Rinse with PBSTw a few times
Block with blocking solution (PBSTw/0.5%TritonX100/10%DMSO/1%goat serum/ 5%BSA) for 2-3hr
Incubate with primary antibody (dilution determined empirically); if double staining on GFP transgenic background also include + 1/500 mouse anti-GFP antibody (Santa Cruz, sc-9996) – note this only works here if other primary is NOT of mouse origin. Incubate in blocking solution at 4 °C for O/N (longer incubation such for 2 overnights makes the staining intense)

<2nd day>
Wash with PBSTw RT for 20min x 5 times
1/1000 goat anti-rabbit IgG-HRP; include 1/500 goat anti-mouse IgG-Alexa488 if co-staining for GFP, in blocking solution at 4 °C for O/N

<3rd day>
Wash with PBSTw for 20min x RT 5 times
Rinse with PBS once
1/50 Tyramide-Cy3 in 1x amplification diluent RT for 3hr in dark room
Wash with PBSTw/0.1%TritonX100 O/N to remove residual TSA-Cy3 from embryos