

MPO stain (Erika Mittge)

1. Fix O/N 4% paraformaldehyde in 1 PBS
2. Rinse the fixed fish 3X 15 min in 1ml 1X PBS + 1% Tween 20 on a rocker at room temperature.
3. Prepare Trizmal buffer (SIGMA 903C-50ml, comes with MPO staining kit)
 - a. WEAR GLOVES
 - b. Make 50ml at a time
 - c. Dilute 1:10 with ddH₂O (eyeball)
 - d. Prewarm to 37°C
4. Add 2ml Trizal mix to peroxidase indicator reagent from kit.
5. Mix, add to 50ml conical of trizal mix.
6. Add 200ul of 3% H₂O₂.
7. Vortex.
8. Add 2ml of mix to fish (fill tubes).
9. Incubate at 37°C 30 min.
10. Rinse with 1X PBS X2.
11. Refix with O/N 4% paraformaldehyde in 1 PBS

TRIZAL WASTE GOES INTO WASTE CONTAINER.
Kit: Leukocyte peroxidase, SIGMA, cat # 390-A