

AQUATIC ZEBRAFISH CORE

PFA

PFA is hazardous if it comes in contact with skin or eyes, and fumes can be toxic. Prepare stock solution in fume hood, wear PPE, including safety glasses while transferring it into a 50ml tube. Use hood when pipetting and opening tube. (see MSDS for further info).

Making PFA

- Our PFA comes as a 16% stock solution in water (#15710, Electron Microscopy Sciences). It is contained in a glass vial with sealed top.
- To open vial wrap in paper towel and break top off in fume hood.
- Empty 10ml content in 50ml tube, tap glass tube carefully to release all liquid.
- Make 40ml 4% PFA in 1X PBS (add in this order).
 - 10ml 16% PFA (stock)
 - 26ml water
 - 4ml 10x PBS

Fixing Fish with 4% PFA

It is important to add cold PFA to the fish and put them on ice to euthanize them instantaneously. **4% PFA must be handled in fume hood!**

- Defrost tube of 4% PFA in water bath, put on ice.
- Anesthetize larvae by adding 2ml Tricaine to dish, scoot larvae to middle of dish with needle (add more tricaine if necessary) (Tricaine (stock 4mg/ml) is in door of fridge in the hallway).
- Transfer fish into 1.5ml tube, let sit in rack for 2-5 minutes (most fish sink to bottom of tube).
- Remove most of embryo medium in fume hood.
- Add 1ml of cold 4% PFA to larvae, mix, put on ice for 5 minutes.
- Leave on rotating platform overnight at 4°C.