

Alcian blue (cartilage) and von Kossa (bone) staining combined

von Kossa:

- Fix fish 1.5h in 4%PFA RT
- Rinse 3times 5min with H2O-Tween
- Add 1ml of 1% silver nitrate solution and sit for 1hour under 60W bulb
- Rinse 1 time 5min with H2O-Tween
- Fix in 2.5% sodiumthiosulphate for 10min
- Rinse 3 times 5min with H2O-Tween
- Fix fish 1.5h in 4%PFA RT
- Bring into MeOH (30%, 60% and 2X 100%)
- Keep fish for 1 hour at RT or store at -20C if necessary
- Reverse MeOH series before carrying on

Alcian blue:

- Stain fish with 180mM Mg AB-solution for 2-4h at RT
- Rinse 1 time 5min with H2O-Tween
- Digest with Trypsin (0.5mg/ml) for 2h at 37C in 40% saturated tetraborate solution (in H2O). Invert periodically. Over night digestion is also possible
- Rinse 3 times 5min with H2O-Tween
- Bring into Glycerol (25%, 50% and 75%)

Notes:

- 1) To avoid pigmentation use PTU treatment or pigment free strain. With nacre fish it makes sense to remove the eyes before AB staining because the pigments leak.
- 2) von Kossa is not as sensitive as Alizarin red stain at early stages
- 3) Fish can not be stored fixed before von Kossa staining
- 4) Unlike Alizarin red, this stain can be stored permanently in 75% glycerol
- 5) Trypsin treat until eyes and brain have dissolved

