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Sudan Black staining of zebrafish larvae

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Abstract

Stains zebrafish larvae with Sudan Black B, which marks neutrophil granules.

Materials

MATERIALS

- ✕ Phenol
- ✕ Ethanol 100%
- ✕ Sudan black B **Merck MilliporeSigma (Sigma-Aldrich) Catalog #199664**
- ✕ Tween20
- ✕ 16% Formaldehyde, methanol-free **Fisher Scientific Catalog #NC1040701**
- ✕ Phosphate buffered saline powder, pH 7.4, for preparing 1 L solutions **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3813**
- ✕ Potassium hydroxide pellets
- ✕ Hydrogen Peroxide, 30% **Fisher Scientific Catalog #H325-500**

Before start

You will need a rocker/nutator/rotator at 4°C and at RT.

Make both 1x and 10x stocks of PBS from powder packets, in water.

Make 70% Ethanol solution.

Make PBS-Tween = 1x PBS + 0.1% (v/v) Tween20.

Make 10% (w/v) Potassium hydroxide (KOH) in water.

Make Sudan Black B stock and working solution:

Stock: 0.18% (w/v) Sudan Black B in 70% Ethanol (store at RT, good for 1+ yr)

Working solution: 10 ml stock + 40 ml 70% Ethanol + 50 µl Phenol (store at RT, good for 6+ mo)

Anesthetize larvae

1. Anesthetize larvae with Tricaine.
2. Transfer to 1.5 ml microcentrifuge tube.

Fix larvae

2. 1. Make **fresh** 4% formaldehyde in PBS:
 - 1 ml 16% formaldehyde
 - 400 μ l 10x PBS
 - 2.6 ml H₂O
2. Pipet as much liquid off of larvae in tubes as possible.
3. Add 1 ml 4% formaldehyde per tube.
4. Cover in aluminum foil, rock at 4°C overnight.

Stain larvae

3. 1. Wash larvae in 1 ml 1x PBS, >5 min, rocking at 4°C.
2. Repeat for a total of 3 washes.
3. Pipet as much PBS as possible off of larvae, add 1 ml working solution of Sudan Black B (see Guidelines- Before you start).
4. Cover in aluminum foil, rock at RT for 1 hour.

Wash and Depigmentation

4. 1. Wash larvae in 1 ml 70% ethanol, 5 min, rocking at RT.
2. Repeat for a total of 3 washes.
3. Rehydrate larvae by washing in PBS-Tween, 5 min, rocking at RT.
4. Pipet as much PBS-Tween as possible off larvae, add 1 ml Depigmentation solution:
 - 400 μ l 10% KOH
 - 133 μ l 30% H₂O₂
 - 3.467 ml H₂O
5. Rock at RT for 5 minutes.
6. Take out a few larvae from tube, check depigmentation under a zoomscope. Pigment should be mostly lost, and sudan black signal should remain.
7. Wash larvae in 1 ml PBS-Tween, 5 min, rocking at RT.
8. Repeat for a total of 2 washes.
9. Store at 4°C. For longer-term storage change liquid to 1x PBS (no Tween).