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Protocol status: Working

Sudan Black staining of zebrafish larvae

PLOS Pathogens

Emily Rosowski¹

¹University of Wisconsin - Madison

Emily Rosowski

ABSTRACT

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

MATERIALS

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

BEFORE START INSTRUCTIONS

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)

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Anesthetize larvae

- 1
 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).