**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**

- **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

**MANUSCRIPT CITATION:**

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)

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 μl 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**

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 µl 10% KOH
   - 133 µl 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 4ºC. For longer-term storage change liquid to 1x PBS (no Tween).