

# Sudan Black staining of zebrafish larvae

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## Abstract

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

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## 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)

## Materials

✓ Phenol by Contributed by users

✓ Ethanol 100% by Contributed by users

🧪 Sudan black B 199664 by Sigma Aldrich


✓ Tween20 by Contributed by users

🧪 16% Formaldehyde, methanol-free  
NC1040701 by Fisher Scientific

🧪 Phosphate buffered saline powder, pH 7.4,  
for preparing 1 L solutions P3813 by Millipore

Sigma

✓ Potassium hydroxide pellets by Contributed by users

 Hydrogen Peroxide, 30% H325-500 by Fisher Scientific

## Protocol

### Anesthetize larvae

#### Step 1.

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

### Fix larvae

#### Step 2.

1. Make **fresh** 4% formaldehyde in PBS:
  - 1 ml 16% formaldehyde
  - 400 µl 10x PBS
  - 2.6 ml H<sub>2</sub>O
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

#### Step 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

#### Step 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 µl 10% KOH

- 133  $\mu$ l 30%  $\text{H}_2\text{O}_2$
- 3.467 ml  $\text{H}_2\text{O}$

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).
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