# Oil Red O staining for fresh frozen skeletal muscle section.v1

**Protocol:**

1. Take out the slides from freezer (-80ºC), thaw it @RT and air dry for 5 min.
2. Fix the slides in 3.7% formaldehyde for 1 hour @ RT.
3. Rinse the slides in deionized water 3 x 30 sec.
4. Stain the slides with Oil red O working solution for 30 min at RT.
5. Rinse the slides in deionized water 3 x 30 sec, and then rinse with running tape water for 5 min.
6. Wipe off the slide, add a drop of 10% glycerol solution, cover with the section with a coverslip and seal it with nail polish.
7. Keep slides in the dark @ 4 ºC and take fluorescent images within 3 days.

**Reagents:**

**3.7% formaldehyde solution:**

Dilute the 37% stock solution (Sigma, F8775) with deionized water.

**Oil Red O stock solution:**

500 mg oil red O (Sigma, O0625) to 100 ml 60% triethyl-phosphate (made with deionized water using 100% solution from Sigma, 90530).

**Oil red O working solution:**

Prior to staining, prepare a 36% triethyl phosphate working solution, containing 12 ml oil red O stock solution and 8 ml deionized water. Filter this solution through #42 Whatman paper to remove crystallized oil red O.

**Embedding medium:**

10% glycerol solution in PBS

**Reference:**

René Koopman, Gert Schaart, Matthijs K.C. Hesselink. Optimization of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem Cell Biol (2001) 116:63–68