Nile Red Staining of Drosophila Larval Tissues

Elizabeth Allen

Abstract

This protocol is used to stain late larval *Drosophila* lipid droplets in fat bodies and intestines with Nile Red, which emits fluorescence in the 552/636 nm range.

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Guidelines

In brief, dissect animals in ice cold phosphate-buffered saline (PBS). Keep tissues in PBS on ice while obtaining your desired sample size. Fix tissues in 4% PFA (diluted in PBS with 0.1% Triton X-100), for 20 minutes, wash 3x in PBS, and stain tissues light-protected at room temperature for 1 hour.

* Do not use any serums for this protocol because Nile Red will instead be drawn away from your tissues and into serum.

Before start

1. Prepare Nile Red (Sigma-Aldrich) in acetone (1000 ug/mL).

2. Dissect tissues in ice cold PBS.

3. Fix tissues in 4% paraformaldehyde (formalin) diluted in PBS with 0.1% Triton X-100.

4. Wash tissues 3x in PBS.

Materials

- 1X PBS (Phosphate-buffered saline) by Contributed by users

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- Triton-X100 by Contributed by users
- Paraformaldehyde Powder (PFA) P6148 by Contributed by users

Nile Red N3013 SIGMA by Sigma Aldrich

Acetone solution 48358 SUPELCO by Sigma Aldrich

Protocol

Step 1.

Dissect tissues in ice cold PBS, keeping samples on ice until required sample size is obtained.

REAGENTS
- 1X PBS (Phosphate-buffered saline) by Contributed by users

Step 2.

Fix tissues in 4% PFA for 20 minutes.

DURATION
00:20:00

Step 3.

Wash tissues 3x in PBS.

REAGENTS
- 1X PBS (Phosphate-buffered saline) by Contributed by users

Step 4.

Stain samples in Nile Red at 0.5 ug/mL diluted in PBS for 1 hour.

DURATION
01:00:00

PROTOCOL

. Nile Red prepared in acetone

CONTACT: Elizabeth Allen

Step 4.1.

Prepare a concentrated working solution of Nile Red in acetone at 1000 ug/mL.

REAGENTS

Nile Red N3013 SIGMA by Sigma Aldrich

Step 4.2.

Store concentrated Nile Red solution at 4°C in the dark for up to 3 months.

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Step 4.3.

Use Nile Red/acetone concentrate diluted in PBS at a concentration of 0.5 ug/mL.

*adjust duration of staining according to the tissue type, and stain at room temperature in the dark.

Step 5.

Wash tissues 3x in PBS.

REAGENTS

- 1X PBS (Phosphate-buffered saline) by Contributed by users

Step 6.

Carefully replace the PBS with mounting medium before transferring samples to slides and imaging.

* Image immediately, or temporarily store slides at 4°C.

PROTOCOL

. Mounting Media for Immunohistochemistry - Drosophila

CONTACT: Sonia Hall

Step 6.1.

90% glycerol
10% 1M Tris-base pH 8.0
0.5% n-propyl-gallate

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