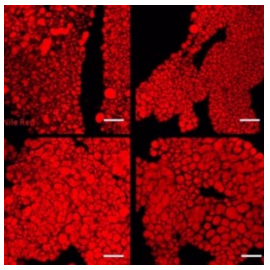
Jul 29, 2019

Nile Red Staining of Drosophila Larval Tissues



Forked from Nile Red Staining of Drosophila Larval Tissues

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Protocol status: Working
We use this protocol and it's working

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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.

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.

Materials

MATERIALS

- 1X PBS (Phosphate-buffered saline)
- Triton-X100
- Paraformaldehyde Powder (PFA) **Catalog #**P6148
- Nile Red **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N3013 SIGMA
- Acetone solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**48358 SUPELCO

STEP MATERIALS

- 1X PBS (Phosphate-buffered saline)
- 1X PBS (Phosphate-buffered saline)
- 1X PBS (Phosphate-buffered saline)

Protocol materials

- Acetone solution **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**48358 SUPELCO
- 1X PBS (Phosphate-buffered saline)
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- 1X PBS (Phosphate-buffered saline)
- Triton-X100
- Paraformaldehyde Powder (PFA) **Catalog #**P6148
- Nile Red **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N3013 SIGMA
- 1X PBS (Phosphate-buffered saline)
- 1X PBS (Phosphate-buffered saline)
- 1X PBS (Phosphate-buffered saline)

Troubleshooting

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.

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

1X PBS (Phosphate-buffered saline)

2 Fix tissues in 4% PFA for 20 minutes.

00:20:00

3 Wash tissues 3x in PBS.

1X PBS (Phosphate-buffered saline)

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

01:00:00

Protocol

NAME

Nile Red prepared in acetone

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Elizabeth Allen

Preview

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

Nile Red **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**N3013 SIGMA

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

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.

5 Wash tissues 3x in PBS.

1X PBS (Phosphate-buffered saline)

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

NAME

Mounting Media for Immunohistochemistry - Drosophila

CREATED BY

Sonia M Hall

Preview

6.1 90% glycerol
10% 1M Tris-base pH 8.0
0.5% n-propyl-gallate