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Squalene Quantification using Nile Red Staining (Developmental)



Forked from [Squalene Quantification using Nile Red Staining \(M4455 Version\)](#)

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Protocol status: In development

We are still developing and optimizing this protocol

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Keywords: squalene quantification, using nile red staining, squalene, nile red staining, fluorescent dye, dye, nile red, hydrophobic substance, stain, cell membrane, lipid vesicle, vesicle

Abstract

This protocol is under development!

Nile Red is a fluorescent dye that stains selectively hydrophobic substances. We assume that squalene accumulates in the cell membrane or in lipid vesicles where it can be stained by the dye.

Guidelines

This protocol is under development and for teaching purposes only!

Materials

MATERIALS

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

 DMSO Merck MilliporeSigma (Sigma-Aldrich) Catalog #D1435

Before start

This protocol is under development and for teaching purposes only!

Culture Sampling

- 1 Sample **1-2 ml** *Synechocystis* culture, measure its OD at **750 nm** and adjust it to **2 ml of OD (750 nm) = 0.5** in BG-11 media. Split your adjusted culture in **2 × 1 ml**. One portion will be stained with Nile Red, the other will serve as a negative control.

Staining

- 2 Stain one portion of the previously adjusted culture with **c_(final) = 5 µg/ml Nile Red in DMSO**. Add the same volume of DMSO to the unstained control samples.

Safety information

Wear gloves and a lab coat when working with DMSO as it stains skin and cloth.

Washing

- 3 **Incubate** your cultures for **60 min** in the dark.
 01:00:00 Incubation
- 4 **Centrifuge** all samples for **5 min at 8000 xg**.
 00:05:00 Centrifugation
- 5 Carefully **discard** the supernatant by pipetting. Avoid resuspending the pellet or losing cells! Try removing as much supernatant as possible.
- 6 Thoroughly **resuspend** all pellets in **1 ml BG-11 media**, **centrifuge** again (**5 min at 8000 xg**), **discard** the supernatant and again **resuspend** the pellet in **1 ml BG-11 media**.
 00:05:00 Centrifugation
- 7 Optional: Keep the removed supernatant to check the quantity of free Nile Red in the supernatant! Ideally, you can repeat the washing steps until no more Nile Red is present in the supernatant.

Data Gathering



- 8 Fill each **200 μ l** of your cultures in 96 well plate's wells. Your sample volume should allow four wells per sample.
- 9 Measure each well's **OD(750 nm)** in the plate reader.
- 10 Measure Nile Red fluorescence. Use following wavelenghts: **Excitation: 510 nm/Emission: 660 nm**
- 11 Analyze your data: Normalize each well's fluorescence to the respective OD and subtract fluorescence without Nile Red from fluorescence with Nile Red. Compare your results to wild type Nile Red stained fluorescence.