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In devel.

Squalene Quantification using Nile Red Staining (Developmental)

Forked from Squalene Quantification using Nile Red Staining (M4455 Version)

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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.

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

GUIDELINES

This protocol is under development and for teaching purposes only!

MATERIALS

NAME	CATALOG #	VENDOR
Nile Red	N3013 SIGMA	Sigma Aldrich
DMSO	D1435	Sigma Aldrich

BEFORE STARTING

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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 **2x 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 culures 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 supernatand 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 µl** 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.