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🌐 Squalene Quantification using Nile Red Staining (M4455 Version)

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Protocol status: In development

We are still developing and optimizing this protocol. It is for teaching only!

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ABSTRACT

This protocol is under development and for teaching purposes only!

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

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⊗ Nile Red Sigma Aldrich Catalog #N3013
SIGMA

⊗ DMSO Sigma Aldrich Catalog
#D1435

BEFORE START INSTRUCTIONS

This protocol is under development and for teaching purposes only!

- 1 Sample **1-2 ml** *Synechocystis* culture, measure its OD at **750 nm** and adjust it to **2 ml of OD (750 nm) = 0.2** 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.

- 2 Stain one portion of the previously adjusted culture with $c_{\text{(final)}} = 10 \mu\text{g/ml}$ Nile Red.

Safety information

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

- 3 Incubate your cultures for **30 min** in the dark.

00:30:00 Incubation

- 4 Centrifuge all samples for **5 min at 5000 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**.

- 7 Fill each **200 μl** in 96 well plate's wells. Your sample volume should allow four wells per sample.

- 8 Measure each well's **OD(750 nm)** in the plate reader.

- 9 Measure Nile Red fluorescence. Use following wavelengths: **Excitation: 510 nm/Emission : 660**

nm

- 10 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.