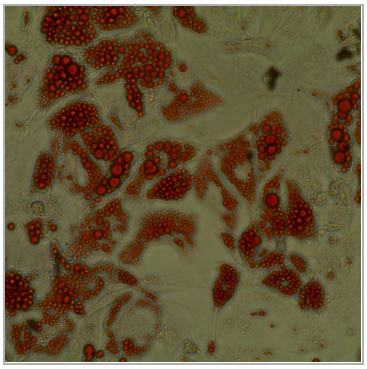

JUN 20, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvo3n9bv4o/v1

Protocol Citation: Laura.SabioRodriguez 2023. Lipid (Oil Red O) Staining . **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo3n9bv4o/v1

MANUSCRIPT CITATION: Lipid (Oil Red O) Staining Kit. SIGMA-ALDRICH, Catalog Number: MAK194

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

Created: Jun 20, 2023

Last Modified: Jun 20, 2023

PROTOCOL integer ID: 83716

Lipid (Oil Red O) Staining

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ABSTRACT

Lipid droplets (LDs) are dynamic, ubiquitously present lipid-storage organelles, predominantly present in the adipocytes. Triglycerides, neutral lipids, and cholesterol esters stored in LDs are the largest sources of energy. The presence of excess LDs in adipocytes results in obesity and obesity-linked pathologies such as dyslipidemia and diabetes type.

ATTACHMENTS

Lipid Oil Red O Staining kit.pdf

SAFETY WARNINGS

Hazard Classifications
Acute Tox. 3 Inhalation - Acute Tox. 4 Oral - Carc. 1B - Eye Irrit. 2 - Muta. 2 - Skin Irrit. 2 - Skin Sens. 1 - STOT SE 3

Keywords: Lipid droplets, Staining, Adipocytes, Oil Red O

BEFORE START INSTRUCTIONS

Kit: **Lipid (Oil Red O) Staining Kit. SIGMA-ALDRICH, Catalog Number: MAK194**
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

Reconstitute the Oil Red O Stock solution with 20 mL of Isopropanol (IPA) 100% **Contributed by users**. Mix well and leave undisturbed for 00:20:00 to make the Stock Solution, which is stable for 1 year.

The Oil Red O Working Solution is obtained by adding 3 parts of Oil Red O Stock Solution to 2 parts of water. Mix well and leave undisturbed for 00:10:00 and filter through Whatman No. 1 filter paper. The Working Solution is stable for 2h and must be prepared 15 min before use.

Procedure

30m

1 All components:

Use 100 µL / well for a 96 well plate

Use 2 mL / well for a 6 well plate

Use 6 mL / well for a 100 mm culture dish

2 **Fixation** 30m

Remove the medium and gently wash the cells twice with 1X PBS (Phosphate-buffered saline). Add 10 % formalin **Contributed by users** to the cells and incubate for 00:30:00 to 1 h.

Note: Do not add formalin directly onto the cells. Pipette onto the wall and mix gently rotating.

3 5m

Discard the formalin and wash the cells twice using Distilled Water **Contributed by users**.

Add 60% Isopropanol **Contributed by users** to the cells and incubate for 00:05:00.

4 Discard 60% isopropanol and cover the cells evenly with **Oil Red O Working Solution**. Rotate the 10m

plate or dish, and incubate for 00:10:00 to 20 min .

5 Discard the Oil Red O Solution and wash the cells 2-5 times with Distilled Water **Contributed by users** until no excess stain is seen.

6 Add Hematoxylin **Contributed by users** to the cells and incubate for 00:01:00 . 1m

Discard hematoxylin and wash the cells 2-5 times with Distilled Water **Contributed by users**

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7 Cover the cells with Distilled Water **Contributed by users** and view under microscope. Lipid droplets appear red and nuclei appear blue.

Note: Keep the cells covered with water to avoid drying.