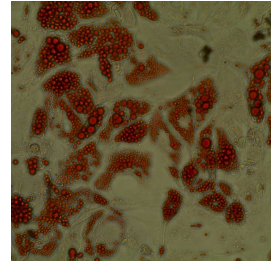


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Lipid (Oil Red O) Staining

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

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Protocol status: Working

We use this protocol and it's working

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Keywords: Lipid droplets, Staining, Adipocytes, Oil Red O, lipid droplet, lipid, present lipid, triglyceride, neutral lipid, present in the adipocyte, adipocyte, adipocytes result, cholesterol ester, organelle, obesity, diabetes

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 Stai...](#)

226KB

Protocol materials

✕ Isopropanol (IPA) 100%

✕ 1X PBS (Phosphate-buffered saline)

✕ 10 % formalin

✕ Distilled Water

✕ Isopropanol

✕ Hematoxylin



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

Before start

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% . 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 μ L / 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 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 Discard the formalin and wash the cells twice using Distilled Water . Add 60%

5m

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

10m

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

6 Add Hematoxylin to the cells and incubate for 00:01:00 . Discard hematoxylin and wash the cells 2-5 times with Distilled Water .

1m

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

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