Procedures for the staining of lipid droplets with Oil Red O

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Abstract

Goal:

This document aims to standardize the protocol for the staining of lipid droplets with Oil Red O (ORO), and subsequent observation using fluorescence microscopy.

The technique of staining with Oil Red O can be used to observe changes in the lipid metabolism of animal cells, caused by different agents such as bacteria or drugs (such as statins), hormones (such as IGF-1), and several small molecules (such as PGL-1). Excessive accumulation of lipids is the key feature of several metabolic events and / or diseases, so identifying and quantifying these changes are essential for a better understanding of these physiological events. These lipid droplets are stained by Oil Red O.

Field of application:

Oil Red O is a lipid-soluble lysochrome ($C_{26}H_{24}N_4O$) with a maximum absorption at 518 nm. This dye is used for the staining of neutral lipids (triglycerides and diacylglycerols), in addition to cholesterol esters, but does not bind to biological membranes. The principle of this technique is based on the poor solubility of the ORO in the solvent, and the solubility is further decreased by the dilution of ORO in water prior to use. In this way, the hydrophobic dye will move from the solvent to associate with the lipids within tissue sections.

One limitation of this technique is the inability to differentiate the labeled lipid species. This dye does not label polar lipids (i.e., phospholipids, sphingolipids and ceramides).

General considerations:

- Cells to be analyzed should be cultured on 13 mm coverslips in 24-well plates

- Oil Red O labeling does not need to be carried out sheltered from the light and most of the steps can be carried out outside the biological safety booth, since the material will be fixed with 4% paraformaldehyde.

- The reagent is 0.05% Oil Red O solution in 85% propylene glycol.

- Weigh the Oil Red O powder. Take a funnel and place a coffee filter into the funnel. Then place the Oil Red O powder inside the filter and pour the volume of Propylene Glycol 85% into the filter, calculated for the 0.05% solution. Wait for the solution to pass through the filter (this may take a few minutes). If Propylene Glycol 85% stock has run out, Propylene Glycol pro-analysis should be diluted in distilled water to a concentration of 85%.

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- This protocol is organized in 3 steps: Fixation of the cells with 4% paraformaldehyde (PFA) and staining with Oil Red O, nuclear staining with DAPI and assembly of the slide.

Experimental proceedings:

Part 1 – Fixation of cells and staining of lipid bodies (droplets):

1. Remove the culture medium inside the safety cabinet (BSC) with the aid of a pipette and add 250 µL of 4% PFA per well. Incubate for 20 minutes by supporting the plate inside a styro foam with ice;

2. Remove the 4% PFA with a pipette and add 250 µL of 1 x PBS, dripping it slowly on the walls of the wells. Remove the PBS with a pipette, without leaning the tip on the bottom of the plate. Repeat this wash.* From this step on, the protocol can be performed outside the BSC.

3. Add 250 µL of propylene glycol pro-analysis to each well, incubate for 7 minutes at room temperature;

4. Remove the propylene glycol with a pipette but do not wash;

5. Add 250 µL of 0.05% Oil Red O solution. Incubate for 5 minutes at room temperature;

6. Remove the propylene glycol with a pipette but do not wash;

7. Remove the propylene glycol with a pipette but do not wash;

8. Remove the previous solution with a pipette and add 250 µL of 1x PBS, dripping it slowly on the walls of the wells. Remove the PBS with a pipette, without leaning the tip on the bottom of the plate. Repeat this wash 3 times.

Part 2 - Nuclear Staining with DAPI:

1. Dilute the DAPI according to the instruction set in the reagent box (dilute 100x) with distilled water). The final volume of DAPI is related to the number of wells that will be analyzed;

2. Add 250 µL of DAPI to each well and incubate for 3 minutes at room temperature. When staining the cells with DAPI it is necessary that the plate remains in a dark environment, thus, you should cover the plate with laminated paper so that there is no interference of light.

3. Remove the DAPI solution with the aid of a pipette and add 250 mL of 1x PBS, dripping it slowly on the walls of the wells. Remove the PBS with a pipette, without leaning the tip on the bottom of the plate. Repeat this wash 3 times.

Part 3 – Slide assembly:

1. Identify the slides with the conditions to be analyzed and drip 3 µL of mounting medium (antifade) on the slides.

2. Then remove the coverslip from the well and, with the aid of forceps, place it inverted on the slide, so that the cells are in contact with the antifade.

3. To seal the slide Entellan mounting medium or colorless nail polish is used.

OBS_1: In the washing steps, 1x PBS should be slowly added to the wall of the well, this will serve to ensure that the fixed cells do not release from the plate.

OBS_2: The mounting medium (Entellan) is very viscous. Thus, it is useful to make a small cut at the tip of the pipette tip so that manipulation with Entellan becomes easier.

References:

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Annika Mehlem, Carolina E Hagberg, Lars Muhl, Ulf Eriksson & Annelie Falkevall. Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease. *Nature Protocols* 8,1149–1154 (2013)

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