Oil Red O Staining for Cultured Cells

1. Culture and treat cultured cells in tissue culture plate as needed (see other protocols).
2. Take the plate (35-mm) out of incubator and remove the medium.
3. Add ~2 ml of PBS to wash the cells and remove PBS completely.
4. Add 2 ml of 10% formalin (RT) and incubate for 10 min at RT.
5. Discard formalin and add 2 ml fresh formalin. Incubate for at least 1 hour, or longer (Cells can be kept in formalin for a couple of days before staining. Wrap with parafilm and cover with aluminum foil to prevent cells from drying).
6. Remove formalin with a pipette.
7. Wash cells with 2 ml of ddH2O twice.
8. Wash cells with 2 ml of 60% isopropanol for 5 min at RT.
9. Let the cells dry completely at RT. If possible, use a hairdryer to dry.
10. Add 1 ml of Oil Red O working solution and incubate at RT for 10 min.
11. Remove Oil Red O solution and **immediately** add ddH2O. Wash the cells 4 times with ddH2O.
12. Acquire images under the microscope for analysis.
13. Remove all the water and let dry.
14. Elute Oil Red O dye by adding 1 ml of 100% isopropanol and incubate for 10 min with gently shaking.
15. Pipet the isopropanol with Oil Red O up and down several times to ensure that all Oil Red O is in the solution.
16. Transfer the solution to a 1.5-ml eppendorf tube.
17. Measure OD at 500 nm using100% isopropanol as blank.

Reagents

1. Oil Red O Stock: Sigma (Cat# O-0625), FW 408.5. Weigh 0.35 g Oil Red O and put in 100 ml of isopropanol. Stir O/N, filter (0.2 μ) and store at RT.
2. Oil Red O Working Solution: Mix 6 ml of Oil Red O stock solution with 4 ml of ddH2O. Let sit at room temp for 20 min followed by filtering (0.2 μ).
3. 10% Formalin in PBS: Dilute 27 ml of formalin stock solution (37%, Merck, Cat# K36658003) in 63 ml of ddH2O and 10 ml of 10X PBS.
4. 100% Isopropanol (Merck, Cat# K36543834)
5. 60% Isopropanol: Mix 6 ml of 100% Isopropanol with 4 ml of ddH2O.

Summary

Sudan III, Oil red O and Sudan black are lysochromes (fat soluble dye) predominantly used for demonstrating triglycerides in frozen sections. Oil Red O has largely replaced sudan III and sudan IV as it is much deeper red in color, and consequently more clearly visible.

Originally written by Lenka Janderova