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Oil Red O Stain For *In Vitro* Adipogenesis

Materials

- DPBS- 1X without calcium and magnesium
- 10% formalin, neutral buffered – VWR catalog # VW3239-4 or equivalent
- Oil red o – Sigma catalog # 0-0625 or equivalent
- 99% isopropanol – Sigma catalog # 1-0398 or equivalent
- 60% isopropanol
- Hematoxylin – Harris Sigma catalog # HHS-16 or equivalent
- Induced adipogenic cultures
- Conical filter paper – Whatman No. 1 or equivalent

Procedure

Fixing adipogenic cultures

1. Remove cells from incubator and place in the fume hood. All procedures involving formalin must be performed in a fume hood.
2. Aspirate the media first off the control and then off the induced wells of the post-induction adipogenic plate.

NOTE: Be sure not to let the cells remain dry for longer than 30 seconds at any point during the fixation or staining processes.

3. Gently rinse the plate with 2 mL of sterile DPBS along the sides of each well of the adipogenic plate so as not to disturb the monolayer.
4. Aspirate the DPBS and add 2 mL of 10% formalin along the sides of each well of the plate, again being careful not to disrupt the cells.
5. Incubate the plates for at least 30 minutes or up to 1 hour at room temperature.

Preparing oil red o stain

1. Prepare the stock solution by weighing out 300 mg of oil red o powder and adding this to 100 mL of 99% isopropanol. This solution is stable for one year from the date on which it is prepared.
2. In the fume hood, mix 3 parts (30 mL) of oil red o stock solution with 2 parts (20 mL) deionized water and allow to sit at room temperature for 10 minutes. The working solution is stable for no longer than 2 hours, so make up only what will be used in that time.
3. Place a piece of filter paper in a funnel and place the funnel in a 125 mL plastic bottle.
4. Filter the oil red o working solution completely through the filter funnel. Add the solution slowly so as not to overflow the funnel.

Staining adipogenic cultures

1. Slightly tilting the plate, remove the formalin from the sides of each well with a pipettor and discard the formalin into a designated formalin waste receptacle. Remove the formalin from the control wells first.
2. Gently add 2 mL of sterile water to each well to rinse.
3. Remove the water with a pipette and discard the water in the formalin waste receptacle.
4. Add 2 mL of 60% Isopropanol to cover the bottom of each well and let sit for 2-5 minutes.
5. Pour off the Isopropanol and pipet 2 mL of the working solution of oil red o along the side of each well so that the cells are completely covered.
6. Slowly rotate the dish to spread oil red o evenly over the cells and then let stand for 5 minutes.
7. Rinse with tap water down the center of each plate until the water runs clear. Be sure not to add running water directly to the wells because this may disrupt the monolayer.
8. Pipette 2 mL of the hematoxylin counterstain into each well so that the cells are completely covered and let stand for 1 minute.
9. Pour the hematoxylin off and rinse the plate with warm tap water as in the above steps.
10. Keep the plates wet with water until they are ready to be viewed.
11. View the plates on a phase contrast microscope. Lipids will appear red and the nuclei will appear blue.

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References:

Pittenger MF, Mackay AM, Bech SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143-147 Novikoff AB, Novikoff PM, Rosen OM, Rubin CS (1980) J. Cell Biol. 87:180-196