ADIPOCYTE STAINING WITH OIL RED O

REAGENTS

Oil Red O stock
FW 408.5, Sigma O-0625
0.7 g Oil Red O
200 ml Isopropanol
Stir O/N, then filter with 0.2 µm and store at +4°C

Oil Red O Working Solution
6 parts Oil Red O stock
4 parts dH_2O
Mix and let sit at room temp for 20 min
Filter 0.2 µm

10% Formalin in PBS

Isopropanol 100%
Isopropanol 60%

METHOD

- Remove most of the medium
- Add 10% formalin in incubate 5 min, RT
- Discard formalin and add the same volume of fresh formalin. Incubate at least 1 hour, or longer. *Note: Cells can be kept in formalin for a couple of days before staining. Wrap parafilm around the plate to prevent from drying and cover with aluminum foil.*
- Remove all the formalin with small transfer pipette
- Wash wells with 60% isopropanol.
- Let the wells dry completely
- Add Oil Red O working solution for 10 min (do not touch walls of the wells)
- Remove all Oil Red O and IMMEDIATELY add dH_2O, wash with H_2O 4 times (you can wash under running tap water)
- Take picktures if desired
- Remove all water and let dry
- Elute Oil Red O by adding 100% isopropanol, incubate about 10 min (can be longer)
- Pipet the isopropanol with Oil Red O up and down several times to be sure that all Oil Red O is in the solution
- Transfer to 1.5 ml tubes
- Measure OD at 500 nm, 0.5 sec reading
- As blank use 100% isopropanol. As control use isopropanol from empty well stained as previously described

Plate	Formalin	60% isopropanol	Oil Red O	100% isopropanol
24WP	500µl	500µl	200µl	750µl
12WP	1ml	1ml	400µl	1.5ml
6WP	2.4ml	2.4ml	1ml	3.6ml

Revised 3/17/05 M. McNeil
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