Lipid Staining Kit
200 Point Assay Kit in 96-well Format
Cat# ST-R100

INSTRUCTION MANUAL ZBM0012.01

STORAGE CONDITIONS

- **Reagents & Buffers:** Room temperature 25°C

For *in vitro* Use Only

For Research Use Only. Not For Use In Diagnostic Procedures

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ORDERING INFORMATION AND TECHNICAL SERVICES

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INTRODUCTION

This protocol is designed to stain cells in a 96-well format using Oil Red O. Oil Red O is a fat-soluble diazo dye that stains neutral triglycerides and lipids. For other formats, please adjust the volumes added to each well according to the surface area of the well/flask you are using.

ITEMS INCLUDED IN THE KIT

ITEM	STORAGE NOTES	UNIT	QTY
Fixative Solution	Store at room temperature, preferably in a cabinet approved for storage of flammable chemicals.	40 ML BOTTLE	1
Oil Red O Stock Solution	Store at room temperature in a dark bottle, preferably in a cabinet approved for storage of flammable chemicals.	6 ML BOTTLE	1
Syringe	10 cc syringe	EACH	1
Filter	1μm PTFE filter	EACH	1
Tray	For multi-channel pipettes, clear polyvinyl	EACH	3

Other equipment/reagents required but not provided with the kit:

- Mature adipocytes or other lipid-containing cells to be stained
- Deionized or distilled water (dH_2O)
- Multi-channel Pipet , single channel pipette and pipette tips
- Tubes for preparing working solutions

ASSAY PROCEDURE

96-well plate format Protocol

1. Remove row by row all the liquid and add 100μl/ well Fixative Solution. Incubate at room temperature for 5 minutes. Repeat liquid exchange with another 100μl/ well Fixative Solution.
2. Fix cells at least 1 hour at 4°C. Alternatively, the plate of cells in Fixative Solution may be sealed using sealing film/foil (not included) and stored at 4°C for 7 days before proceeding.
3. **Prepare Working Oil Red Solution (WOROS): (Prepared fresh on day of assay) 40% water / 60% Oil Red O stock.**
    a. Add 6.0 ml of Oil Red O Stock Solution to 4.0 ml of water and **mix by inversion**.
    b. This working solution must be kept at room temperature at least 20 minutes before filtering.
    c. Filter out particulate material using a 1μm polypropylene PTFE syringe filter (supplied with the kit) or #1 Whatman filter paper (not provided). NOTE: Filter paper is effective; it just takes longer to filter. Polycarbonate filters will dissolve in isopropanol; DO NOT USE THEM.
4. Remove all the Fixative Solution. Wash cells at least twice with 200 μl/well dH_2O using a multichannel pipette. Remove all the water using a multichannel pipette. Make sure all the water is removed **BEFORE** proceeding.
5. Add 50 μl/ well Working Oil Red O solution (WOROS) at room temperature for 10-15 minutes. Be careful not to touch the sides of the wells; the pipette tips should go at a 90° angle into the wells.
6. Remove all of the Oil Red O solution from each well. Wash 2-3 times with 60-80 μl/ well dH_2O. Pictures may be taken at this time. Leave water inside wells during photography.