Lipid Staining Kit

Cat# ST-R100

INSTRUCTION MANUAL (ZBM-12)

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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- **World Wide Web** **http://www.zen-bio.com**

Items included in the kit:

The protocol is designed for assay of cells in a 96-well format. For other formats, please adjust the volumes added to each well according to the surface area of the well/flask you are using.

ITEM	STORAGE NOTES	UNIT	QTY
Fixative Solution	Store at room temperature, preferably in a cabinet approved for storage of flammable chemicals.	40ML 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.	6ML BOTTLE	1
Syringe	10 cc syringe	EACH	1
Filter	1μm PTFE filter	EACH	1
Tray	For multi-channel pipetters, 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 pipet and pipet tips

Assay Procedure

96-well plate format Protocol

1. Remove row by row all the liquid and add 100μl/ well Fixative Solution. Allow the plate to set at room temperature for about 5 minutes. Repeat liquid exchange with another 100μl/ well Fixative Solution.
2. Cells must be fixed for at least 1 hour at 4°C. Alternately, cells in Fixative Solution may be sealed using sealing film/foil and kept at 4°C for at least 7 days before proceeding.
3. **Prepare Working Oil Red Solution (WOROS): (Prepared fresh on day of assay) Prepare a working solution 40% water and 60% Oil Red O stock. Mix by inversion.**
    a. This working solution must be kept at room temperature at least 20 minutes before filtering.
    b. Filter out particulate material using a 1μm polypropylene PTFE syringe filter (supplied with the kit) or #1 Whatman filter paper. 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 at least twice using a multichannel pipetter by adding 200 μl/well dH_2O. Remove all the water using a multichannel pipetter. 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 pipet tips should go at a 90° angle into the wells.
6. Remove all the Oil Red O solution from each well. Wash 2-3 times with 60-80μl/ well dH_2O. Pictures may be taken at this time. Leave water inside wells during photography.