BIOMEDICAL RESEARCH SERVICE CENTER
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Adipogenesis Assay Kit (Cat #: A-106)

COMPONENTS: Oil Red Solution- 100 ml, store at room temperature (for 200 assays)

PRODUCT DESCRIPTION: Adipogenesis is associated with upregulation of many adipocyte-specific genes, culminating in the synthesis of fatty acids which are stored in the cytosol as triacylglycerols. These lipid molecules are water insoluble and coalesce to form fat globules visible to the naked eyes. The Oil Red dye is hydrophobic displaying a distinct deep red color. This dye is commonly used in staining protocols to demonstrate the presence of intracellular lipids and lipid-bound proteins. Since the synthesis of lipids is increased during adipogenesis, staining with Oil Red is a fast and easy method to identify cells undergoing adipogenesis. The lipid-bound Oil Red is readily identifiable by microscopy, and can be extracted with an organic solvent after staining and washing, which then allows one to assay adipogenesis quantitatively by spectroscopy. The assay can be set up in a microplate format.

PROTOCOL:

Cell staining (Although the protocol is optimized for cells grown on 35-mm tissue culture dishes, it can be modified for various experimental settings)

1. Aspirate off culture medium, and wash cells twice with normal phosphate-buffered saline (PBS). Remove PBS solution completely.
2. To each 35-mm dish, add 0.5 ml Oil Red solution for cell fixing and staining. Wait 10 min. You should be able to visualize under microscope stained dark red oil droplets in the cytosol after 10 min. The incubation time can also be increased.
3. Aspirate off solution, and rinse cells twice with distilled water. Remove water completely.
4. For quantitative analysis, add 0.5 ml 60% isopropanol to each 35-mm dish to extract the Oil Red dye. Gently agitate dishes at room temperature for 30 min.
5. Transfer enough dye extracts to a cuvette or a 96-well microplate. Measure optical density at 492 nm ($O.D._{492\ nm}$) using 60% isopropanol as blank. The amount of Oil Red extracted from the cells indicates the extent of adipogenesis.

Tissue staining

The Oil Red Solution can also be used to stain deparaffinized tissue sections. Soak slides in the dye solution for 30-60 min followed by washing with distilled water.

NOTE:

- Avoid skin contact with the Oil Red Solution, which contains 60% isopropanol. Please note that isopropanol is flammable. Please visit the product webpage for MSDS information on isopropanol.
- Recipe for PBS solution: 2.68 mM KCl, 137 mM NaCl, 1.47 mM KH_2PO_4, & 8.06 mM Na_2HPO_4.