BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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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 and exhibits 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, providing a quantitative assay by absorbance at 490-520 nm. The Oil Red Solution is stable for many years if handled properly.

PROTOCOL:

Cell staining:

The protocol is optimized for monolayer cells grown on 35-mm culture dishes. However, it can be modified for various cell culture settings.

1. Aspirate off culture medium from culture dish. Gently rinse cells twice with normal phosphate-buffered saline (PBS). Aspirate off PBS solution completely. Note: *No cell fixation is necessary.*

2. To each 35-mm dish, add 0.5 ml Oil Red solution and wait ~10 min at room temperature. Check cells under an inverted microscope to make sure red oil droplets are visible. The incubation time can also be increased.

3. Aspirate off solution, and rinse cells three times with distilled water. Remove water completely. Cells are ready for imaging.

4. For quantitative analysis, add 0.5 ml 60% isopropanol to each 35-mm dish. Gently agitate dishes at room temperature for ~30 min.

5. Transfer 0.2 ml of each isopropanol extract to a cuvette or a plain (uncoated) 96-well plate. Measure optical density at 490-520 nm using 60% isopropanol as blank. The amount of Oil Red extracted from the cells indicates the extent of adipogenesis.

Tissue section staining:

1. Use paraffin-embedded tissue sections for the staining. Deparaffinize tissue sections first.

2. Immerse slides in Oil Red Solution for ~30 min followed by rinsing with distilled water three times. The tissue sections are ready for imaging.

NOTE:

- Avoid skin contact with the Oil Red Solution, which contains 60% isopropanol. Note that isopropanol is flammable. Please visit the product webpage for material SDS information on isopropanol and Oil Red.