Intracellular Lipid Quantitation Kit

For the staining and quantitative determination of lipids in adipocytes.

Cat. No. KT-101

For Research Use Only. Not for use in Diagnostic Procedures.
PRODUCT INFORMATION
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PRINCIPLE
The K-ASSAY® Intracellular Lipid Quantitation Kit is for the staining and quantitative determination of lipids in adipocytes. Oil Red O is a lipophilic dye that indicates adipogenesis by staining intracellular lipid droplets red. Lipids are quantified by organic extraction of intracellular lipids. The K-ASSAY® Intracellular Lipid Quantitation Kit includes Oil Red O, fixative, and lipid extraction solution to quantify lipids in adipocytes. For research use only. Not for use in diagnostic procedures.

STORAGE
Store kit components at room temperature.

COMPONENTS

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil Red O Stock Solution</td>
<td>2x 150 mL</td>
</tr>
<tr>
<td>Extraction Solution</td>
<td>2x 200 mL</td>
</tr>
</tbody>
</table>

Kit Size: Enough reagent for staining 30 x 24-well plates.

MATERIALS REQUIRED BUT NOT PROVIDED
10% formalin or reagents to prepare fixative solution described below
Pipette
0.5–1.0 µm syringe filter
Graduated cylinders
Microplate reader or spectrophotometer capable of reading absorbance at 540 nm.
Distilled water
Phosphate buffered saline (PBS)

PRECAUTIONS
1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only, not for human or diagnostic use.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.

REAGENT PREPARATION

Fixative Solution:
37% Formaldehyde 100 mL
Distilled Water 900 mL
NaH2PO4(H2O) 4 g
Na2HPO4 6.5 g

Prepare Oil Red O working solution before use. Mix Oil Red O Stock Solution with distilled water at a ratio of 6:4. Let the mixture stand at room temperature (RT) for 10-15 min. Filter the Oil Red O Working Solution with a 0.5–1.0 µm syringe filter.

Note: The working solution is stable for no longer than 2 hours. The solution containing crystallized Oil red O cannot be used.

PROTOCOL
1. Remove culture medium from adipocytes grown in 24-well plates. Wash each well with 500 µL of PBS.
2. Add 500 µL of the Fixative to each well and let stand for 15 minutes at RT.
3. Wash each well three times with 500 µL of distilled water.
4. Add 500 µL of the filtered Oil Red O Working Solution to each well. Let stand at RT for 15 minutes.

5. Remove Oil Red O Working Solution and wash each well at least three times with distilled water or until water is clear. Water has to be clear after washing.

6. If the background color is too red, it may interfere with quantitation. Allow the wells to air dry.

7. Add 500 µL of Extraction Solution to each well to extract the dye.

8. Shake the plate on an orbital shaker for a few minutes or until the bound dye is extracted.

9. Measure the OD (540 nm) with a plate reader or spectrophotometer.

Example of Lipid Quantitation of Mesentery Adipocytes

Effects of Culture Media on Differentiation of Mesentery Adipocytes

<table>
<thead>
<tr>
<th>Culture Media</th>
<th>OD (540 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>B</td>
<td>1 ± 0.1</td>
</tr>
<tr>
<td>C</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>D</td>
<td>2 ± 0.2</td>
</tr>
<tr>
<td>E</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>F</td>
<td>3 ± 0.3</td>
</tr>
<tr>
<td>G</td>
<td>4 ± 0.4</td>
</tr>
<tr>
<td>H</td>
<td>4 ± 0.4</td>
</tr>
</tbody>
</table>

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