Oil Red O Staining Kit
Specifications and Instruction Quick Steps

Oil Red O Staining Kit Components
Oil Red O stains lipid vesicles which are created by functional adipocytes and is a useful tool when used with Lifeline’s mesenchymal stem cells and Lifeline’s AdipoLife™ Adipogenesis Medium Complete Kit. This kit contains fixative solution, dehydration solution, Oil Red O stain solution and stain differential solution for staining lipid vesicles in adipocytes.

Safety Statement
These products are For Research Use Only and are not approved for human or veterinary use, or for use in in vitro diagnostics or clinical procedures. Paraformaldehyde releases formaldehyde gas which is a known carcinogen. It is best to work with paraformaldehyde in a chemical fume hood. Please dispose of paraformaldehyde in accordance with local and federal regulations.

<table>
<thead>
<tr>
<th>Product</th>
<th>Part No.</th>
<th>Volume</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil Red O Lipid Staining Kit</td>
<td>LL-0052</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5% Oil Red O Stain Solution</td>
<td>CM-0054</td>
<td>100 mL</td>
<td>RT</td>
</tr>
<tr>
<td>4% Paraformaldehyde Fixative Solution</td>
<td>CM-0055</td>
<td>100 mL</td>
<td>-20°C</td>
</tr>
<tr>
<td>100% 1,2-Propanediol Dehydration Solution</td>
<td>CM-0056</td>
<td>100 mL</td>
<td>RT</td>
</tr>
<tr>
<td>85% 1,2-Propanediol Stain Differential Solution</td>
<td>CM-0057</td>
<td>100 mL</td>
<td>RT</td>
</tr>
<tr>
<td>Other Recommended Products</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Human Mesenchymal Stem Cells-Adult (HMSC-Ad)</td>
<td>FC-0034</td>
<td>10⁶ Cells</td>
<td>-150°C</td>
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<tr>
<td>StemLife™ MSC Medium Complete Kit</td>
<td>LL-0034</td>
<td>Kit</td>
<td></td>
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<td>AdipoLife™ Adipogenesis Medium Complete Kit</td>
<td>LL-0050</td>
<td>Kit</td>
<td></td>
</tr>
<tr>
<td>Phosphate Buffered Saline</td>
<td>CM-0001</td>
<td>500 mL</td>
<td>RT</td>
</tr>
</tbody>
</table>

Deionized water, coarse filter paper

The Lifeline® Guarantee
Lifeline’s rigorous quality control ensures performance to standardized testing criteria. If Lifeline’s products do not meet our posted performance and quality standards, we will replace them at no charge or provide a full refund. Upon request, Lifeline will provide lot-specific QC test results, material safety data sheets and certificates of analysis. See complete guarantee/warranty statement at lifelinecelltech.com or contact your Lifeline representative for more information.
Lifeline's adult mesenchymal stem cells differentiated to adipocytes and stained with Oil Red O (200X).

Quick Steps for Fixation and Staining of Adipocytes for Lipid Vesicles in 6-Well Plates

1. Always wear eye protection and gloves when working with staining reagents.
2. Use 4% Paraformaldehyde Fixative Solution in a chemical fume hood.
3. Adipocytes may be stained with Oil Red O at either 37°C or 60°C.
4. Pre-heat an oven to 60°C or a water bath to 37°C.
5. For each well (6-well plate) to be stained, pre-warm 2.5 mL of Oil Red O (37°C or 60°C) in a 50 mL conical tube.
6. Once Oil Red O has been warmed, filter through paper towel or coarse Whatman filter paper into a fresh tube to remove small particulates. Return Oil Red O to warming at 37°C or 60°C.
7. Partially aspirate medium from well. Never allow monolayer to be exposed to air at any point during staining. If the wells are allowed to dry the lipid vesicles will burst.
8. Gently, from the side of the well, add 2 mL of Lifeline® PBS (CM-0001).
9. Repeat Steps 5 and 6 two more times.
10. Partially aspirate PBS from the wells.
11. Add 2 mL of 4% Paraformaldehyde Fixative Solution (CM-0055) to each well of a 6-well plate.
12. Partially remove 4% Paraformaldehyde from each well, and replace with another 2 mL of 4% Paraformaldehyde.
13. Fix for at least 20 minutes at room temperature.
15. Rinse well twice with deionized water.
16. Add 1.0 mL of 100% 1,2-Propanediol Dehydration Solution (CM-0056) to each well and incubate for 5 minutes at room temperature. Gently tilt the plate 2 to 3 times to mix.
17. Partially remove dehydration solution. Add another 1 mL of 100% 1,2-Propanediol Dehydration Solution to each well and incubate for 5 minutes at room temperature. Gently tilt the plate 2 to 3 times to mix.
18. Partially remove dehydration solution. Add 2 mL Oil Red O Stain Solution (CM-0054) to each well.
19. Incubate at 37°C for 30 minutes or at 60°C for 8 minutes. Tilt the plate 2 to 3 times during the incubation.
20. Remove Oil Red O. Add 2 mL of 85% 1,2-Propanediol Stain Differential Solution (CM-0057) for 1 minute to differentiate stain. Do not mix the stain differential solution in the well(s)!
21. Rinse wells carefully twice with 2 mL dH2O per well, leaving the final dH2O rinse in the well.

For any question on cell handling, differentiation or staining, please contact technical service. We are here to help.

Call Lifeline Technical Service and Sales at 877.845.7787
or visit lifelinecelltech.com for more information