Flow-cytometric determination of neutral lipids

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SCOPE OF THE METHOD

<table>
<thead>
<tr>
<th>The Method relates to</th>
<th>Human health</th>
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<tbody>
<tr>
<td>The Method is situated in</td>
<td>Translational - Applied Research</td>
</tr>
<tr>
<td>Type of method</td>
<td>In vitro - Ex vivo</td>
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<tr>
<td>Specify the type of cells/tissues/organs</td>
<td>Human skin-derived adults stem cells</td>
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</tbody>
</table>

DESCRIPTION

Method keywords
Flow-cytometry
neutral lipids
quantitative
fluorimetric
in vitro

Scientific area keywords
Steatosis
stem cells
lipids
lipid accumulation

Method description
Using this method you can measure the (relative) lipid load in human skin-derived stem cells differentiated towards hepatic cells. This method could also be applied on other cell types (e.g. HepG2), since it is based on the following publication: "M. T. Donato et al., Chem. Biol. Interact. 181, 417–423 (2009)." Briefly: 1. Aspirate medium from the cell culture 2. Incubate 10′ with TrypLE (200 µL/well for 24- multiwell format) 3. Add 500 µL pre-warmed PBS (37°C) to every well and harvest the sample 4. Rinse with 500 µL PBS 5. Centrifigate according to the cell type 6. Resuspend in 1 mL PBS (containing BODIPY(TM) 1:2500 (see publication above)) on ice 7. 5′ before measuring + 1 µL Hoechst (+homogenize by pipetting) 8. Dilute 1:10 (PBS (4 °C)) before measuring to limit background signal 9. Measure signal (up to 100,000 events)

Lab equipment
Flow-cytometer;
Cell culture equipment;
Biosafety cabinet.

Method status
Internally validated

PROS, CONS & FUTURE POTENTIAL

Advantages
Fast.

Challenges
Measuring many samples can be time-consuming.

**Modifications**

You can use also other cell types.

**Future & Other applications**

Drug testing (anti-steatotic drugs);
Assessing drug-induced liver steatosis (*in vitro*).

**REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION**

**References**