

# Flow-cytometric determination of neutral lipids

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## Organisation

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**Specific Research Group or Service** In Vitro Toxicology and Dermato-Cosmetology

**Country** Belgium

**Geographical Area** Brussels Region

## SCOPE OF THE METHOD

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

## 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. Centrifuge 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**

M. T. Donato et al., Chem. Biol. Interact. 181, 417–423 (2009)

R. M. Rodrigues et al., Stem Cells Dev. 23, 44–55 (2014)

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