Flow-cytometric determination of neutral lipids

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Organisation

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SCOPE OF THE METHOD

The Method relates to	Human health
The Method is situated in	Translational - Applied Research
Type of method	In vitro - Ex vivo
Specify the type of cells/tissues/organs	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. 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

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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