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Cellular lipid uptake with flow cytometry readout

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Igor Beletchi[1], rosanne.wouters[1], Peter Vangheluwe[1]
[1]KU Leuven

rosanne.wouters

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We use this protocol and it's working

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ASAP (Aligning Science Across Parkinson's)

Abstract

Cellular lipid uptake with flow cytometry readout

Protocol materials

- TrypLE Express or preferred cell dissociation reagent **Thermo Fisher Scientific**
- PBS without Ca2 or Mg2 **Gibco - Thermo Fisher Scientific Catalog #**10010-031
- HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112
- NBD-SM (C6 NBD sphingomyelin) **Avanti Polar Lipids, Inc. Catalog #**810218C
- NBD-GluCer (C6-NBD Glucosyl Ceramide) **Avanti Polar Lipids, Inc. Catalog #**810222C
- NBD-PC (18:1-06:0 NBD PC) **Avanti Polar Lipids, Inc. Catalog #**810132C
- NBD-lyso-PC (12:0 lyso NBD PC) **Avanti Polar Lipids, Inc. Catalog #**810128C
- NBD-PS (18:1-06:0 NBD PS) **Avanti Polar Lipids, Inc. Catalog #**810194C
- NBD-PE (18:1-06:0 NBD PE) **Avanti Polar Lipids, Inc. Catalog #**8105155C
- Albumin Bovine Serum Fraction V Fatty Acid-Free **Merck Catalog #**126575

harvest cells

10m

1 harvest cells by detachment with
TrypLE Express or preferred cell dissociation reagent **Thermo Fisher Scientific**

1.1 cells should be grown to 70% confluency, not higher

1.2 wash cells with
TrypLE Express or preferred cell dissociation reagent **Thermo Fisher Scientific**

1.3 incubate cells with
TrypLE Express or preferred cell dissociation reagent **Thermo Fisher Scientific**
(3ml/T175 flask) at room temperature

1.4 when cells detach resuspend with 7ml
PBS without Ca2 or Mg2 **Gibco - Thermo Fisher Scientific Catalog #**10010-031
and keep on ice in 15 mL falcon

2 collect cells by centrifugation 400 x g, 4°C, 00:05:00

5m

3 wash pellet with with
PBS without Ca2 or Mg2 **Gibco - Thermo Fisher Scientific Catalog #**10010-031

4 collect cells by centrifugation 400 x g, 4°C, 00:05:00

5m

5 resuspend pellet in HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112

6 count cells

7 prepare cell suspension with 1*10^6 cells in 500µl
HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112

prepare lipid suspension

8 work under fume hood equipped with N2 flow

9 wash glass syringe 3x with 100% ethanol and 3x with chloroform, let dry

10 with glass syringe pipette needed volume of NBD-lipid into glass vial
NBD-PC (18:1-06:0 NBD PC) **Avanti Polar Lipids, Inc. Catalog #**810132C ,
NBD-lyso-PC (12:0 lyso NBD PC) **Avanti Polar Lipids, Inc. Catalog #**810128C ,
NBD-PS (18:1-06:0 NBD PS) **Avanti Polar Lipids, Inc. Catalog #**810194C ,
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10.1 keep glass bottle with NBD-labeled lipids in chloroform solution cold, by working on ice or in freezer block

10.2 for each sample 500µl lipid suspension with 2µM final concentration is needed

11 dry lipids by evaporating chloroform under N2 flow

12 resuspend lipids in HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112 to final concentration of 2µM with magnetic stirbar until fully dissolved, keep lipids at 4°C while dissolving

lipid uptake

13 equilibrate cell suspension 0 rpm, 37°C, 00:15:00

14 equilibrate lipid suspension 0 rpm, 37°C, 00:15:00

15 prepare collection tubes with 200µl HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112 containing 5% Albumin Bovine Serum Fraction V Fatty Acid-Free **Merck Catalog #**126575 (per sample 1 collection tube is needed for each timepoint) and keep on ice

16 for timepoint 0, take 200µl of equilibrated cell suspension and add to collection tube T=0 (containing 200µl of ice-cold HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112 containing 5% Albumin Bovine Serum Fraction V Fatty Acid-Free **Merck Catalog #**126575) on ice

17 add 500µl of lipid suspension to remaining 300µl of cell suspension

18 incubate at 37°C with thermoshaker with interval 1 min shaking every 5 min (700 rpm).

18.1 keep samples away from light during incubation

19 after 30min, take 200µl of lipid+cell suspension and add to collection tubes T=30 (containing 200µl of ice-cold HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112 containing 5% Albumin Bovine Serum Fraction V Fatty Acid-Free **Merck Catalog #**126575), keep on ice

20 after 60min, take 200µl of lipid+cell suspension and add to collection tubes T=60 (containing 200µl of ice-cold HBSS **Gibco - Thermo Fisher Scientific Catalog #**14170-112 containing 5% Albumin Bovine Serum Fraction V Fatty Acid-Free **Merck Catalog #**126575), keep on ice

21 add sodium dithionite to all samples (1/100 dilution of freshly prepared 1M stock in tris-buffer, pH10) and vortex samples (make sure to mix well with quencher)

22 measure total internal fluorescence with flow cytometer

Protocol references

The lipid uptake assay was adapted from (Naito *et al.*, 2015).