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Cellular lipid uptake with flow cytometry readout

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ABSTRACT

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Protocol status: Working We use this protocol and it's working

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10m harvest cells 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

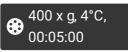
2

1.4

collect cells by centrifugation (3)

15 mL falcon

when cells detach resuspend with 7ml



PBS without Ca2 or Mg2 Gibco, ThermoFisher Catalog #10010-031

5m

and keep on ice in

wash pellet with with PBS without Ca2 or Mg2 Gibco, ThermoFisher Catalog #10010-031

- resuspend pellet in HBSS Gibco Thermo Fischer Catalog #14170-
- 6 count cells
- 7 prepare cell suspension with 1*10^6 cells in 500μl

 HBSS Gibco Thermo Fischer Catalog #14170-

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
 - \bowtie 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

 NBD-SM (C6 NBD sphingomyelin) Avanti Polar Lipids, Inc. Catalog
 #810218C

 NBD-GluCer (C6-NBD Glucosyl Ceramide) Avanti Polar Lipids, Inc. Catalog
 #810222C
- 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
- dry lipids by evaporating chloroform under N2 flow
- resuspend lipids in HBSS Gibco Thermo Fischer Catalog #14170to final
 concentration of 2μM with magnetic stirbar until fully dissolved, keep lipids at 4°C while dissolving

lipid uptake

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

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