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Untargeted lipidomics of Tagless Lyso-IP

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We use this protocol and it's working

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Abstract

Lysosomal biology is increasingly implicated in neurodegenerative diseases and health. It has traditionally been difficult to profile the metabolomic homeostasis of the lysosome in disease states. To overcome this challenge we have developed the Tagless Lyso-IP method to rapidly prepare lysosome enriched samples from human peripheral blood. This protocol details the processing and untargeted analysis of nonpolar metabolites derived using the Tagless Lyso-IP method.

Attachments

1023-2636.pdf

87KB

Materials

Reagents

- Water, Optima™ LC/MS Grade, Fisher Chemical™ **Thermo Fisher Scientific Catalog #**AAB-W6-4
- Acetonitrile, Optima™ LC/MS Grade, Fisher Chemical™ **Thermo Fisher Scientific Catalog #**AAB-A955-4
- Isopropanol, Optima™LC/MS Grade, Fisher Chemical™ **Thermo Fisher Scientific Catalog #**AAB-A461-500
- Ammonium formate
- Formic acid
- EASYIC™
- Splashmix (SPLASH® LIPIDOMIX® Mass Spec Standard, cat. no. 330707)
- Sodium chloride 0.9% in aqueous solution Normal saline solution, sterile **Avantor Sciences Catalog #**S5825 (101320-574)

Equipment

- ID-X Orbitrap Tribrid Mass Spectrometer (Thermo Fisher Scientific) with a heated electrospray
- ionization (HESI) probe

Equipment	
Ascentis® Express C18, 2.7 µm HPLC Column	NAME
HPLC Column	TYPE
Ascentis®	BRAND
53825-U	SKU
https://www.sigmaaldrich.com/IN/en/product/supelco/53825u	LINK

Equipment

Ascentis® Express Guard Cartridge Holder	NAME
for use with Ascentis Express Guard Columns, pk of 1	TYPE
Ascentis®	BRAND
53500-U	SKU
https://www.sigmaaldrich.com/IN/en/product/supelco/53500u	LINK

Equipment

DynaMag™- Spin Magnet	NAME
Invitrogen™	BRAND
12320D	SKU
https://www.thermofisher.com/order/catalog/product/12320D	LINK

- Microcentrifuge with thermostat (VWR Micro Star 17R. S/N 42209232. REF# 521-1647)
- Eppendorf ThermoMixer® C, Eppendorf, #EP02095

Equipment

ThermoTop®	NAME
ThermoTop®	BRAND
5308000003	SKU
https://www.eppendorf.com/us-en/eShop-Products/Temperature-Control-and-Mixing/Accessories/ThermoTop-p-5308000003	LINK

Equipment

Savant™ SpeedVac™ Medium Capacity Vacuum Concentrators for Combinatorial Chemistry Applications	NAME
Thermo Scientific™	BRAND
SPD140DDA-115	SKU
https://www.thermofisher.com/order/catalog/product/SPD140DDA-115	LINK

C18-based lipid separation

Buffer A

	A	B
	Ammonium formate	10 millimolar (mM)
	Formic acid	0.1 % (v/v)
	Dissolve in	
	LC/MS grade water	Dissolve in 60 % (v/v)
	LC/MS grade acetonitrile	Dissolve in 40 % (v/v)

Buffer B

	A	B
	Ammonium formate	10 millimolar (mM)
	Formic acid	0.1 % (v/v)
	Dissolve in	
	LC/MS grade 2-propanol	90 % (v/v)
	LC/MS grade acetonitrile	10 % (v/v)

Troubleshooting

Untargeted lipidomics of Tagless Lyso-IP

1 This method is following successful isolation of lysosomes the Tagless Lyso-IP method as described in: dx.doi.org/10.17504/protocols.io.x54v9yp51g3e/v1 (Tagless Lyso-IP).

> Note
>
> In the steps following the immunoprecipitation of lysosomes (Steps 28-32) the wash buffer used is ice-cold KPBS without protease and phosphatase inhibitors.

Processing of nonpolar metabolite samples (lipids)

2 Resuspend the lysosomes attached to the magnetic beads and the pelleted whole cell samples in 1000 µL of chloroform:methanol at ratio of 2:1 (v/v) with 1000x diluted Splashmix (Avanti).

2.1 Incubate at 4 °C for 00:10:00 . 10m

3 Place the Tagless Lyso-IP samples on a tube magnet for 00:00:30 and transfer the supernatant to a fresh 1.5 mL Eppendorf tube. 30s

4 Vortex both the Tagless Lyso-IP samples and their corresponding whole cell samples (from Step 1) at 4 °C for 01:00:00 . 1h

5 Add 200 µL of 0.9% (w/v) saline (VWR) and vortex at 4 °C for 00:10:00 . 10m

6 Centrifuge all samples at 3000 x g , 4 °C for 00:05:00 . 5m

7 Discard the top layer (MeOH and saline polar phase) and use the bubbling method to retrieve the bottom layer of chloroform containing the lipids to a fresh 1.5 mL Eppendorf tube.

8 Vacuum dry the samples and store at -80 °C.

9 On the day of analysis reconstitute the dried lipid extracts in 50 µL of ACN:IPA:water 13:6:1 (v/v/v).

10 Vortex at 4 °C for 00:10:00. 10m

11 Centrifuge at 13000 x g, 4 °C for 00:15:00. 15m

12 Insert 45 µL of supernatant into glass insert vials for LC/MS.

LC/MS lipidomics settings

13 Set an ID-X tribrid mass spectrometer (Thermo Fisher Scientific) with a heated electrospray ionization (HESI) probe, for initial nonpolar lipid profiling.

14 Prepare an Ascentis Express C18 150 × 2.1 mm column (Millipore Sigma 53825-U) coupled with a 5 × 2.1 mm guard (Sigma-Aldrich 53500-U), to carry out C18-based lipid separation prior to mass spectrometry. Use EASYICTM for internal calibration.

15 For C18-based lipid separation, for buffer preparation refer to the material section.

16 Set the chromatographic gradient flow rate to 0.26 mL/min.

16.1 Use Orbitrap resolution 120,000 for MS1 and 30,000 for MS2.

16.2 Use RF lens at 40%.

16.3 Use AGC target 4×105 for MS1 and 5×104 for MS2.

16.4 Use maximum injection time 50 ms for MS1 and 54 ms for MS2.

16.5 Set positive ion voltage to 3250 V, negative ion voltage to 3000 V, ion transfer tube temperature to 300 °C, and vaporizer temperature to 375 °C.

16.6 Set sheath gas flow to 40 units, auxiliary gas flow to 10 units, and sweep gas flow to 1 unit.

17 Operate the mass spectrometer in full-scan mode with data-dependent tandem mass spectrometry (ddMS2) at m/z 250-1500, with

	A	B
	Cycle time	1.5 sec
	Microscans	1 unit
	Isolation window	m/z 1
	Intensity threshold	1 × 104
	Dynamic exclusion time	2.5 sec

17.1 For HCD fragmentation, use step-wise collision energies of 15%, 25%, and 35%.

18 Perform the elution with a gradient of 00:40:00 : 40m

18.1 From 0–1.5 min isocratically elute at 32% B.

18.2 From 1.5-4 min linearly increase to 45% B.

18.3 From 4-5 min linearly increase to 52% B.

18.4 From 5-8 min linearly increase to 58% B.

18.5 From 8-11 min linearly increase to 66% B.

18.6 From 11-14 min linearly increase to 70%.

18.7 From 14-18 min linearly increase to 75%.

18.8 From 18- 21 min linearly increase to 97% B.

18.9 From 21-35 min hold at 97% B.

18.10 From 35-35.1 min linearly decrease to 32% B.

18.11 From 35.1-40 min hold at 32% min.

Untargeted lipidomics workflow

19 LipidSearch and Compound Discoverer (Thermo Fisher Scientific) were used for unbiased
differential analysis. Lipid annotation was acquired from LipidSearch with the precursor tolerance at 5 ppm and product tolerance at 8 ppm.

20 The mass list from LipidSearch is then exported and used in Compound Discoverer for improved alignment and quantitation.

	A	B
	Mass tolerance	10 ppm
	Minimum and maximum precursor mass	0-5,000 Da
	Retention time limit	0.1-30 min
	Peak filter signal to noise ratio	1.5

	A	B
	Retention time alignment maximum shift	1 min
	Minimum peak intensity	10,000
	Compound detection signal to noise ratio	3
	Isotope and adduct settings	Default values
	Gap filling and background filtering	Default settings

Note

- The MassList Search was customized with 5 ppm mass tolerance and 1 minute retention time tolerance.
- Area normalization was performed by constant median after blank exclusion.