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sample_prep_serum.nan V.1

Forked from a private protocol

In 1 collection

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Christopher Esselman: Protocol review;
Leandro Ponce: Protocol review

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

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Abstract

This is a modified protocol for a protein precipitation method for plasma/serum samples. This protocol was originally proposed by:

Citation

Nagana Gowda GA, Raftery D (2014)
. Quantitating metabolites in protein precipitated serum using NMR spectroscopy. Analytical chemistry.

https://doi.org/10.1021/ac5005103 LINK

See also:

Citation

Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK (2007)
. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts.
Nature protocols.

Citation

Nagana Gowda GA, Raftery D (2019)
. Analysis of Plasma, Serum, and Whole Blood Metabolites Using 1H NMR Spectroscopy.
Methods in molecular biology (Clifton, N.J.).

https://doi.org/10.1007/978-1-4939-9690-2_2 LINK

Materials

1. Chemicals and reagents

- Phosphate buffer in D2O (100 mM, pH 7.4, 1/3 mM DSS-d6)
- Methanol

2. Equipment

- Calibrated micropipettes (100 µL, 200 µL, and 1000 µL)
- Pippette tips
- 1.5-mL Eppendorf tubes
- 5-mm SampleJet NMR tubes (Bruker)
- Centrifuge
- Vortex mixer
- Speed-vac concentrator

1 Day-1/2

1.1 Thaw samples On ice or at 4 °C

1.2 Add 600 µL of 100% cold methanol to 300 µL of samples On ice

- Use 1.5-mL Eppendorf tubes
- Keep methanol cold On ice

1.3 Vortex the samples for 00:00:10

10s

1.4 Incubate the samples at -20 °C for 00:20:00

20m

1.5 Centrifuge the samples at 4 °C at 16000 rcf for 00:30:00

30m

1.6 Transfer the supernatants to new 1.5-mL Eppendorf tubes

1.7 Dry the samples in a speed-vac concentrator

Overnight (time varies depending on the sample condition)

Note

Store dried samples at -80 °C until the following step if needed

2 Day-2/2

2.1 Thaw the samples On ice or in 4 °C

2.2 Add 600 µL of the phosphate buffer to each sample

2.3 Vortex the samples at 4 °C for 00:10:00 10m

2.4 Centrifuge the samples at 4 °C for 00:00:10 10s

2.5 Transfer 580 µL to 5-mm NMR tubes

Note

No stickers/labels on the caps and tubes allowed

Citations

Nagana Gowda GA, Raftery D. Quantitating metabolites in protein precipitated serum using NMR spectroscopy. **https://doi.org/10.1021/ac5005103**

Beckonert O, Keun HC, Ebbels TM, Bundy J, Holmes E, Lindon JC, Nicholson JK. Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts.

Nagana Gowda GA, Raftery D. Analysis of Plasma, Serum, and Whole Blood Metabolites Using 1H NMR Spectroscopy.
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