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NMR Serum Sample Preparation for NMR metabolomics

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We use this protocol and it's working

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Disclaimer

Abstract

This is a modified protocol to conduct NMR metabolomics for serum samples using methanol extraction. This protocol was originally proposed by:

Nagana Gowda, N.G., Raftery,D.Quantitating Metabolites in Protein Precipitated Serum UsingNMR Spectroscopy. Analytical Chemistry 2014,86 (11),5433–5440.

Guidelines

Frozen samples should be thawed in 4 °C cold room or on ice. All sample preparation has to be done in 4 °C cold room or on ice.

Materials

Chemicals and Reagents

- NaH2PO4, Sodium phosphate monobasic anhydrous
- HPO4, Sodium phosphate dibasic
- HCl, Hydrochloric acid
- NaOH, Sodium hydroxide
- D2O, Deuteriumoxide, 99.9 atom % D
- DSS-D6, Sodium 2,2-dimethyl-2-silapentane-5-sulfonate, 98atom % D
- Methanol
- Pooled quality control

Equipment

- alibrated micropipettes (100-µl, 200-µl, and 1000-µl)
- Pipette tips
- Labels
- 250-ml volumetric flask
- Speed-vac concentrator
- Vortex mixer
- Eppendorf centriguge
- 1.5-ml Eppendorf tubes
- 5-mm SampleJet NMR tubes (Bruker)
- Analytical weighing balance
- pH meter

Before start

Before you start, samples should be randomized and include QA/QC and pooled samples for 2D runs. All tubes should be prelabeled.

Phosphate buffer preparation protocol

1 In a 250 ml volumetric flask, dissolve 2321.55 mg of anhydrous NaH_2PO_4 and 802.25 mg of Na_2HPO4 in 200 mL of D2O.

2 Add 833.25 µL of 0.1 DSS-D6 stock solution (1/3 of 1mM DSS when mixed with sample)

3 Adjust the pH to pH 7.0 using HCL or NaOH

4 Adjust the volume to 250 mL with D_2O and mix well

5 Recheck the pH

6 Store the buffer at 4 °C

NMR Serum Methanol Extraction

7 Thaw samples on ice or at 4 °C

8 Add MeOH to samples on ice at a 3:1 methanol-to-sample ratio
1. 750 µL MeOH 250 µL neat sera for pre-challenge samples
2. 1200 µL MeOH 400 µL near sera for post-challenge samples

9 Aliquot 800 µL of sample

10 Vortex the samples and incubate the samples in -20 °C for 00:20:00

11 Centrifuge the samples at 16000 rcf for 00:30:00

12 Transfer the supernatant of each sample to a new tube

13 Dry the samples in a speed-vac concentrator (time depends on the sample condition)

Store the dry pellets in -80 °C if needed

14 Thaw the sample on ice or in 4 °C

15 Add 600 µL of NMR buffer to each sample to resuspend the pellets

16 Vortex the samples for 00:15:00

17 Centrifuge the samples for 00:00:10

18 Transfer 580 µL into 5-mm NMR tube

Protocol references

Nagana Gowda GA, Raftery D. Quantitating metabolites in protein precipitated serum using NMR spectroscopy. Anal Chem. 2014 Jun 3;86(11):5433-40. doi: 10.1021/ac5005103. Epub 2014 May 14. PMID: 24796490; PMCID: PMC4045325.

see also:

Beckonert, O., Keun, H. C., Ebbels, T. M., Bundy, J., Holmes, E., Lindon, J. C., & Nicholson, J. K. (2007). Metabolic profiling, metabolomic and metabonomic procedures for NMR spectroscopy of urine, plasma, serum and tissue extracts. *Nature protocols*, *2*(11), 2692.