

Feb 20, 2026

NMR Serum Sample Preparation for NMR metabolomics

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5rj6jg1b/v1>

Deanna Lanier¹, Mario Uchimiya¹, Arthur Edison¹

¹University of Georgia



Deanna Lanier

Edison Lab - UGA

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5rj6jg1b/v1>

Protocol Citation: Deanna Lanier, Mario Uchimiya, Arthur Edison 2026. NMR Serum Sample Preparation for NMR metabolomics. protocols.io <https://dx.doi.org/10.17504/protocols.io.8epv5rj6jg1b/v1>

Manuscript citation:

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: May 14, 2024

Last Modified: February 23, 2026

Protocol Integer ID: 99677

Keywords: nmr serum sample preparation for nmr metabolomic, nmr metabolomics for serum sample, nmr metabolomic, nmr serum sample preparation, serum usingnmr spectroscopy, using methanol extraction, methanol extraction, metabolites in protein, serum sample, analytical chemistry, spectroscopy, serum

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

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 centrifuge
- 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_2HPO_4 in  200 mL of D_2O .
- 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  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.