sample_prep_urine.nan

orked from a private protocol

NAN KB[1], Mario Uchimiya[2], John Glushka[2], Leandro I Ponce[2], Saraa Al Jawad[2], Laura Morris[2], Arthur Edison[2]

[1]Network for Advanced NMR (NAN); [2]University of Georgia

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Leandro I Ponce: Protocol review;
Saraa Al Jawad: Protocol review

NAN support at UGA

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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 NMR metabolomics for urine samples. This method was originally proposed by:

CITATION

Dona AC, Jiménez B, Schäfer H, Humpfer E, Spraul M, Lewis MR, Pearce JT, Holmes E, Lindon JC, Nicholson JK (2014). Precision high-throughput proton NMR spectroscopy of human urine, serum, and plasma for large-scale metabolic phenotyping.. Analytical chemistry.

LINK

https://doi.org/10.1021/ac5025039

See also:

CITATION

Emwas AH, Luchinat C, Turano P, Tenori L, Roy R, Salek RM, Ryan D, Merzaban JS, Kaddurah-Daouk R, Zeri AC, Nagana Gowda GA, Raftery D, Wang Y, Brennan L, Wishart DS (2015). Standardizing the experimental conditions for using urine in NMR-based metabolomic studies with a particular focus on diagnostic studies: a review.. Metabolomics : Official journal of the Metabolomic Society.

LINK

https://link.springer.com/article/10.1007/s11306-014-0746-7

MATERIALS

1. Chemicals and reagents
   - Phosphate buffer in D2O (1.5 M, pH 7.4, 1.11 mM DSS-d6)

2. Equipment
   - Calibrated micropipettes (100 µL, 200 µL, and 1000 µL)
   - Pippette tips
   - 1.5-mL Eppendorf tubes
   - 5-mm SampleJet NMR tubes from Bruker
   - Centrifuge
   - Vortex mixer

3. Study samples
   - Urine samples

BEFORE START INSTRUCTIONS

This protocol assumes that the original urine samples have >600 µL.

7m 10s

1 Day-1/1

1.1 Thaw samples On ice or at 4 °C

1.2 Centrifuge the samples at 4 °C at 12000 x g for 00:05:00 min 5m

Note

- This step is to remove particles in the samples
- Sample tube and volume specifications vary depending on the study

1.3 Add 60 µL of the phosphate buffer to 540 µL of the supernatant for each sample

- Use 1.5-mL Eppendorf tubes

1.4 Vortex the samples at 4 °C for 00:02:00 2m

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

Note

This step is to remove samples attached to tube caps, and no centrifugation speed specified

1.6 Transfer 590 µL of the supernatant to an NMR tube for each sample

Note

No stickers/labels on the caps and tubes allowed