



Zika_01_SZ_urine_extraction

Project: Zika | Folder: My Folder | Owner: Sicong Zhang

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Description

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Urine Sample Preparation for NMR:

General Guidelines:

- Frozen samples should be thawed in 4°C cold room or on ice
- Check for the minimum volume of all the samples received
- For 48 samples or less use two blanks (NMR buffer containing DSS), one at the beginning and one at the end
- For more than 48 samples use three blanks, at the beginning, middle, and the end of the set
- Total number of QC samples is 10 % of study samples. One QC after the first blank at the beginning and one before the last blank at the end of the run. The rest of the QC samples are randomized with the client samples using excel randomizer

References:

1. Anthony, C., *et al.* Precision High-Throughput Proton NMR Spectroscopy of Human Urine, Serum, and Plasma for Large-Scale Metabolic Phenotyping. *Analytical Chemistry* 2014, **86 (19)**, 9887-9894.

SOP_URN_Chemicals and Reagents

- KH₂PO₄, Potassium dihydrogen phosphate (Fisher Scientific)
- NaN₃, Sodium azide (United States Biochemical)
- KOH, Potassium hydroxide (Fisher Scientific)
- HCL, Hydrochloric acid (Fisher Scientific)
- D₂O, Deuterium oxide, 99.9 atom % D (Cambridge Isotope Laboratories, Inc.)
- DSS-D6, Sodium 2,2-dimethyl-2-silapentane-5-sulfonate, 98 atom % D (Cambridge Isotope Laboratories, Inc.)
- Pooled quality control (QC) urine (Ethanol, drug and nicotine free human urine female, Golden West Biologicals, Inc.)

SOP_URN_Equipment

- Calibrated micropipettes (100 µl, 200 µl, and 1000 µl)
- Pipette tips
- 1.5 ml eppendorf tubes
- 5 mm SampleJet NMR tubes from Bruker
- 100ml volumetric flask
- Eppendorf centrifuge
- Analytical weighing balance
- Vortex mixer
- pH meter
- Labels

SOP_URN_Phosphate Buffer_1.5M

Steps

1	Dissolve 20.4 g of the KH_2PO_4 in 80 ml of D_2O	00:00:00
2	Dissolve 24.9 mg of DSS-D6 (1/9 of 1mM DSS when mixed with sample) and 13 mg of NaN_3 in 10 ml of D_2O	00:00:00
3	Mix the solutions thoroughly using sonication	00:00:00
4	Check the pH of the solution and adjust it to pH=7.4, the solution should be clear after pH adjusted	00:00:00
5	Use a 100 ml volumetric flask and adjust the volume with D_2O and mix well	00:00:00
6	Recheck the pH and store at 4°C	00:00:00

SOP_URN_Sample_Prep

Steps

1	Thaw samples at 4°C cold room or on ice	00:00:00
2	Centrifuge each sample at 3500 rcf for 5 min at 4°C	00:00:00
3	Take 100 μl supernatant from each sample to a pooled-sample tube (15mL falcon tube)	00:00:00
4	Add 60 μl of NMR buffer with 540 μl of an aliquot of samples in a 1.5 ml eppendorf tube	00:00:00
5	Store at 4°C	00:00:00
6	Add 540 μl QC, 540 μl pooled-sample in 1.5 ml tube with 60 μl buffer	00:00:00
7	Centrifuge each sample at 4000 rcf for 5 min at 4°C	00:00:00
8	Transfer 590 μl of supernatant into 5 mm NMR tube	00:00:00
9	Cap the NMR tubes with Bruker caps without holes or use POM balls to close the tube caps	00:00:00
10	Keep NMR samples on ice and transfer to NMR bay to conduct NMR, if any short delay keep them in 4°C refrigerator	00:00:00

Procedure

Results

Attachments:

Linked Resources

(Box)

(Box)

SCM_SOP_Potassium_Phosphate_Urine_NMR (Protocol)