

Sample preparation for Bile Acid Kit measurement

Solvents:

Acetonitril (Merck KGaA, Darmstadt, Germany hypergrade for LC-MS)

Water MiliQ,

Extracting agent - ACN / H₂O (1:1)

Equipment

4 steel balls size M

Eppendorf Tubes 2mL

Tissue slicer (Rettberg, Germany)

Centrifuge (Sigma)

Work steps

To approximately 100 mg of cecum sample four steel balls of size M are added in Eppendorf tubes. Then add for each mg of sample 5 µL of extracting agent. Shake the samples for 10 minutes at 30 Hz in the tissue slicer and centrifuge for 2 minutes at 14000 rpm. 10 µL of supernatant is used for targeted analytics as described in the kit manufactures manual.

For blood sample analysis 10 µL plasma was taken.

Kit measurement

The analysis was performed using the validated Bile Acid Kit (Biocrates Life Sciences, Innsbruck, Austria) as described in Pham et al. 2017 [1]. Data processing is carried out with the provided quantitation method Kit (Biocrates Life Sciences AG, Innsbruck, Austria).

1 Pham HT, Arnhard K, Asad YJ, Deng L, Felder TK, St John-Williams L, *et al.* Inter-Laboratory Robustness of Next-Generation Bile Acid Study in Mice and Humans: International Ring Trial Involving 12 Laboratories. The Journal of Applied Laboratory Medicine 2019;**1**:129-42.