

## **Sample preparation for Bile Acid Kit measurement**

### **Solvents:**

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

Water MiliQ,

Extracting agent - ACN / H<sub>2</sub>O (1:1)

### **Equipment**

4 steel balls size M

Eppendorf Tubes 2mL

Tissue slicer (Rettberg, Germany)

Centrifuge (Sigma)

### **Work steps**

To approximately 100 mg of 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. The supernatant is used for targeted analytics as described in the kit manufacturer's manual.

### **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.