

SAMPLE PREPARATION PROTOCOL

500 μL of samples for metabolite extraction and analysis were obtained from the compartment of the collection device for FOB measurements as indicated in **Figure 1** and were homogenized using a Precellys 24 homogenizer (Bertin Technologies, France) at 6500 rpm for 23 seconds. Then, 200 μL of homogenized feces extracts were collected and transferred to 1.5 mL microtubes. Afterwards, 780 μL of chloroform:methanol ($\text{CHCl}_3\text{:MeOH}$; 2:1) were added. The $\text{CHCl}_3\text{:MeOH}$ used for extraction was spiked with metabolites not detected in unspiked feces extracts as internal standards (IS) [SM (d18:1/16:0), PE (17:0/17:0), PC (19:0/19:0), TAG (13:0/13:0/13:0), Cer (d18:1/17:0), and ChoE (12:0)]. After a brief vortex mixing, samples were incubated 1 hour at -20°C . After centrifugation at $18,000 \times g$ for 5 minutes, 600 μL of the organic phase were collected, dried under vacuum, and reconstituted in 100 μL acetonitrile:isopropanol (ACN:IPA; 1:1), centrifuged ($18,000 \times g$ for 10 minutes), and transferred to vials for UHPLC-MS analysis.

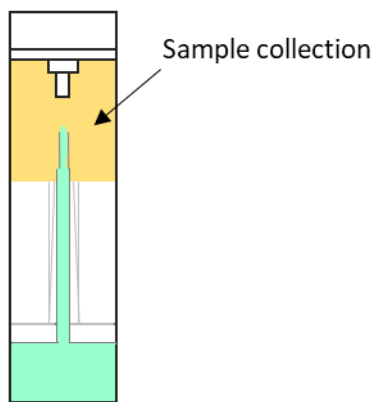


Figure 1. Representation of collection tube and sample collection point.