

This protocol describes digestion of 1 μL raw human blood plasma samples using Sodium deoxycholate and Pierce Trypsin (Thermo Scientific) with products from ProteomEdge.

The protocol has been tested and validated for multiplexed absolute quantification of blood plasma proteins using Mass Spectrometry and products from ProteomEdge.

The panel of protein fragments (qRePS) is located at the bottom of each well in the 96-well plate supplied by ProteomEdge, and can be seen as a white pellet. All wells in a plate are equal with regard to qRePS and amounts.

In our 8-well plates, the first column (A1-H1) holds the panel and in the 48-well plates, the first 6 columns (A1-A6, B1-B6. ...H1-H6) holds the panel.

NOTE: Avoid disrupting the pellet prior to adding your blood plasma samples. It is advised to not pipette samples from the received plate prior to enzymatic digestion.

For optimal performance, follow the protocol or use your own workflow while adding one reagent at the time directly into the wells of the plate.

Consumables

1. Phosphate buffered saline (PBS)
2. Sodium deoxycholate (SDC)
3. Dithiothreitol (DTT)
4. 2-Chloroacetamide (CAA)
5. Pierce Trypsin (Thermo Scientific)
6. Formic acid (FA)
7. Milli-Q ultrapure water (MQ)

Reagents

Volumes below are sufficient for processing one full 96-well plate

- **SDC (350 μL , 10%)**
Dissolve 35 mg SDC in 350 μL MQ
- **DTT (150 μL , 1 M)**
Dissolve 23.1 mg DTT in 150 μL MQ (store at -20°C)
- **DTT + SDC (1000 μL , 30 mM + 3%)**
Mix 30 μL 1M DTT and 300 μL 10% SDC
Add 670 μL MQ
- **CAA (1000 μL , 200 mM)**
Dissolve 18.7 mg CAA in 1000 μL 1x PBS (**keep in the dark**)
- **Trypsin (1000 μL , 0.1 $\mu\text{g}/\mu\text{L}$)**
Dissolve 100 μg Pierce Trypsin in 1000 μL 1x PBS (**keep on ice**)

Procedure

1. Dilute blood plasma samples 10-times using 1x PBS.
Dilute the plasma according to your pipetting accuracy such as 45 μL 1x PBS + 5 μL raw plasma and mix by pipetting up and down
2. Centrifuge the qRePS plate (2000 g, 1 min) and remove the seal
3. Add 10 μL of 1x PBS into the qRePS plate with dried standards.
Use jet dispensing or pipette on the well wall.
NOTE: Do not touch the qRePS pellet with pipette tip!
4. Centrifuge the qRePS plate to get the 1x PBS to the bottom of each well. (2000 g, 1 min)
5. Add 10 μL of diluted plasma to the qRePS plate
Mix by pipetting up and down to dissolve the qRePS pellet
6. Add 10 μL of 30 mM DTT and 3% SDC mix. (Final concentrations 10 mM DTT, 1% SDC)
7. Vortex and centrifuge. (2000 g, 1 min)
8. Incubate at 37°C, 60 min.
9. Protect the samples from direct light and add 10 μL of 200 mM CAA. (Final concentration 50 mM CAA)
10. Vortex and centrifuge. (2000 g, 1 min)
11. Incubate at room temperature **in the dark**, 30 min.
12. Dilute to 0.25% SDC by adding 70 μL 1x PBS
13. Add 10 μL of 0.1 $\mu\text{g}/\mu\text{L}$ Trypsin, vortex and incubate 16 hours over night at 37°C. (Final enzyme:substrate ratio 1:50)
14. Centrifuge. (2000 g, 1 min)
15. Add 10 μL 6.5% FA to quench digestion and let SDC precipitate for 30 min. (Final concentration 0.5% (v/v))
16. Vortex and centrifuge. (3000 g, 15 min)
17. Transfer the supernatant to a new plate to inject directly for LC/MS-MS analysis or perform solid-phase extraction using C18 StageTips or similar.