ProteomEdge
AlbaNova University Center
SE-106 91 Stockholm
SWEDEN

# Protocol

## Digestion with TCEP

This protocol describes digestion of 1 µL raw human blood plasma samples using TCEP/CAA mixture for simultaneous reduction and alkylation at 90°C 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. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl)
2. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl)
3. 2-Chloroacetamide (CAA)
4. Pierce Trypsin (Thermo Scientific)
5. Formic acid (FA)
6. Milli-Q ultrapure water (MQ)

## Reagents

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

- Tris-HCl buffer (50 mL, 50 mM)
  Dissolve 394 mg Tris-HCl in 50 mL MQ

- TCEP-HCl (2000 µL, 30 mM)
  Dissolve 17.2 mg TCEP-HCl in 2000 µL Tris-HCl (store at -20°C)

- CAA (2000 µL, 60 mM)
  Dissolve 11.2 mg CAA in 2000 µL Tris-HCl (**keep in the dark**)

- TCEP-HCl + CAA (4000 µL, 15 mM + 30 mM respectively)
  Mix 2000 µL 30 mM TCEP-HCl with 2000 µL 60 mM CAA

- Trypsin (1000 µL, 0.1 µg/µL)
  Dissolve 100 µg Pierce Trypsin in 1000 µL Tris-HCl (**keep on ice**)

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## Procedure

1. Dilute blood plasma samples 10-times using 50 mM Tris-HCl buffer.
   Dilute the plasma according to your pipetting accuracy such as 45 µL Tris-HCl + 5 µL raw blood 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 Tris-HCl 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 Tris-HCl 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 40 µL TCEP-HCl + CAA mixture. (Final concentrations 10 mM TCEP-HCl, 20 mM CAA)
7. Vortex and centrifuge the samples. (2000 g, 1 min)
8. Incubate at 60°C, 10 min in the darkness.
9. Let the plate cool down to room temperature and centrifuge the plate. (2000 g, 1 min)
10. Add 10 µL of 0.1 µg/µL Trypsin, vortex and incubate 16 hours over night at 37°C. (Final enzyme:substrate ratio 1:50)
11. Centrifuge the plate. (2000 g, 1 min)
12. Add 10 µL 4% FA to quench digestion. (Final concentration 0.5% (v/v))
13. Vortex and centrifuge. (2000 g, 1 min)
14. 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.