

This protocol describes digestion of 1  $\mu\text{L}$  raw human blood plasma samples using RapiGest SF (Waters) and SOLu-Trypsin (Sigma-Aldrich) 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

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1. Phosphate buffered saline (PBS)
2. RapiGest (Waters)
3. Dithiothreitol (DTT)
4. 2-Chloroacetamide (CAA)
5. SOLu-Trypsin (Sigma-Aldrich)
6. Formic acid (FA)
7. Milli-Q ultrapure water (MQ)

## Reagents

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

- **RapiGest (1000  $\mu\text{L}$ , 0.3%)**  
Dissolve 3 mg RapiGest in 1000  $\mu\text{L}$  MQ
- **DTT (150  $\mu\text{L}$ , 1 M)**  
Dissolve 23.1 mg DTT in 150  $\mu\text{L}$  MQ (store at  $-20^{\circ}\text{C}$ )
- **DTT (1000  $\mu\text{L}$ , 30 mM)**  
Dilute 30  $\mu\text{L}$  1M DTT in 970  $\mu\text{L}$  1x PBS
- **CAA (1000  $\mu\text{L}$ , 200 mM)**  
Dissolve 18.7 mg CAA in 1000  $\mu\text{L}$  1x PBS (**keep in the dark**)
- **Trypsin (1000  $\mu\text{L}$ , 0.1  $\mu\text{g}/\mu\text{L}$ )**  
Dilute 100  $\mu\text{L}$  SOLu Trypsin in 900  $\mu\text{L}$  1x PBS (**keep on ice**)

## Procedure

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1. Dilute blood plasma samples 10-times using 1x PBS.  
Dilute the plasma according to your pipetting accuracy such as 45  $\mu\text{L}$  1x PBS + 5  $\mu\text{L}$  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  $\mu\text{L}$  of 0.3% RapiGest into the 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 RapiGest to the bottom of each well. (2000 g, 1 min)
5. Add 10  $\mu\text{L}$  of diluted plasma to the qRePS plate.  
Mix by pipetting up and down to dissolve the qRePS pellet.
6. Add 10  $\mu\text{L}$  of 30 mM DTT. (Final concentrations 0.1% RapiGest, 10 mM DTT)
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  $\mu\text{L}$  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. Add 10  $\mu\text{L}$  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)
13. Centrifuge. (2000 g, 1 min)
14. Add 10  $\mu\text{L}$  3% FA to quench digestion and cleave RapiGest. (Final concentration 0.5% (v/v))
15. Vortex and centrifuge. (2000 g, 1 min)
16. Inject directly for LC/MS-MS analysis or perform solid-phase extraction using C18 StageTips or similar.