HCPprofiler
by ANAQUANT

PROTOCOL
TAKE A BEAD OUT FROM THE VIAL

1. 
Unscrew the cap and place it on top of vial

2. 
Return the vial upside down

3. 
Gently incline the vial while 1 bead stays into the cap

4. 
Transfer the READYBEADS from the cap into your working tube. A tube or a vial is required to optimally dissolve the bead.

PROTOCOL FOR DSP SAMPLES

Step 1

60 µL of sample diluted to **2.5 mg/ml**

Step 2 Denaturation

180 µL of **Urea 8M**

10min at ambient temperature

Step 3 Reduction

27 µL of **DTT 200mM**

40 minutes at 60°C

Step 4 Alkylation

90µL of **IAA 200mM**

40 minutes at ambient temperature

Step 5 Digestion

7.5 µL of **trypsin** at 1mg/mL

QSP 1500 µL with Ammonium bicarbonate 50Mm pH8

Overnight digestion at 37°C
Stop digestion with **1.5 µL** of **TFA**

Step 6 HCPprofiler
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READYBEADS

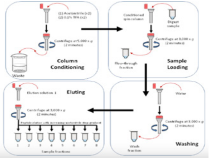
Add **1 beads.**
Agitate 2 minutes for beads dissolution

Centrifugation 15000g for 20min
And supernatant recovery

Step 7 High pH sample fractionation

ANAQUANT recommend to use Pierce fractionation kit (Ref 84868)

Fractionation protocol according to Pierce user guide



1. **Conditioning:** 2X 300µLACN and 2X 300µL 0.1% TFA
Then Centrifuge at 5000g 2 minutes

2. **Sample loading:** 2X300µL of digested sample

Then centrifuge at 3000g 2 minutes

3. **Washing:** 300 µL H2O and centrifuge at 3000g 2 minutes

4. **Elution:** Elute in 8 fractions of 300 µL according to following table

Fraction No.	Acetonitrile (%)	Acetonitrile (µL)	Triethylamine (0.1%) (µL)
1	5.0	50	950
2	7.5	75	925
3	10.0	100	900
4	12.5	125	875
5	15.0	150	850
6	17.5	175	825
7	20.0	200	800
8	50.0	500	500

5. Sample evaporation to dryness

Step 8 Sample injection

Solubilization in **150µL** of starting mobile phases

Step 9 Datas reprocessing

Proteome Discoverer, use:

- FASTA-ProteomeDiscoverer_HCPprofiler
- Parsing rules and layout provided by ANAQUANT
- Export Proteins.txt and PeptideGroups.txt

Contact ANAQUANT for other software use

Step 10 HCPprofiler application

Choose software and import data in HCP application


Import New Sample

Sample name: Sample 1_HCPprofiler_Fractionation

Fractionnement: ● on
BEADS diluted into sample: ○ off

Fraction Final volume per fraction: 150 µL
Injected volume: 5 µL
BEADS volume: 60 µL

Quality control accession - opt: CQ
Injected QC quantity - opt: X fmol

Drug substance (Accession) - opt: DS
Isoelectric Point - opt:
Injected drug substance quantity - opt: 2000 ng

Import Sample →

2/5 of the whole BEADS (=fractionation)

Possibility of adding a quality control before the digestion step
Example with BSA:
- Proline: sp|P02769|ALBU_BOVIN
- Proteome Discovere: P02769

Not necessary with proteome discoverer

STORAGE CONDITIONS

Store your READYBEADS at -20°C in their original vial

For research use only (RUO)

For more information, visit **anaquant.com**