HCPprofiler
by ANAQUANT

PROTOCOL
TAKE A BEAD OUT FROM THE VIAL

1. 

Unscrew the cap and place it on top of vial

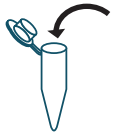
2. 
Return the vial upside down

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.

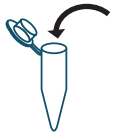
SAMPLE PROTOCOL FOR HARVEST

Step 1



20µL of sample diluted to **1mg/ml**

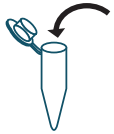
Step 2 Denaturation



60µL of **Urea 8M**

10min at ambient temperature

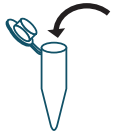
Step 3 Reduction



9µL of **DTT 200mM**

40 minutes at 60°C

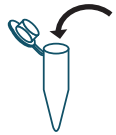
Step 4 Alkylation



30µL of **IAA 200mM**

40 minutes at ambient temperature

Step 5 Digestion



10µL of **trypsin** at 0.1mg/mL

QSP 600µL with Ammonium bicarbonate 50Mm pH8

Overnight digestion at 37°C
Stop digestion with **6µL** of **formic acid 10%**

Step 6 HCPprofiler by ANAQUANT

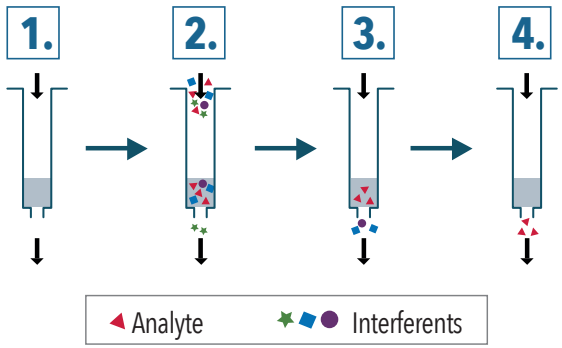
Add **1 beads.**
2 minutes agitation for beads dissolution

Centrifugation 15000g for 20min
And supernatant recovery

Step 7 Sample desalting

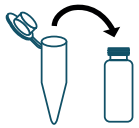
Recommended by ANAQUANT before injection:

Use SPE C18 3cc



1. **Conditioning:**
   1mL MeOH
   then 1mL 0.1%AF in H2O
2. **Sample addition**
3. **Washing:**
   2 x 500 µL 0.1%AF in H2O
4. **Elution:**
   2 x 500 µL 80%MeOH 0.1%AF
5. Sample evaporation to dryness

Step 8 Sample injection



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

Put it in vial

Injection: **2µL**

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

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**