

PROTOCOL TAKE A BEAD OUT FROM THE VIAL

-  Unscrew the cap and place it on top of vial
-  Return the vial upside down
-  Gently incline the vial while 1 bead stays into the cap
-  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



20 μ L of sample diluted to 1mg/ml

Step 2 Denaturation



60 μ L of Urea 8M
10min at ambient temperature

Step 3 Reduction



9 μ L of DTT 200mM
40 minutes at 60°C

Step 4 Alkylation



30 μ L of IAA 200mM
40 minutes at ambient temperature

Step 5 Digestion



10 μ L of trypsin at 0.1mg/mL

QSP 600 μ L with Ammonium bicarbonate 50mM pH8



Overnight digestion at 37°C
Stop digestion with 6 μ L of formic acid 10%

Step 6

HCPprofiler

by ANAQUANT



Add 1 beads.
2 minutes agitation for beads dissolution

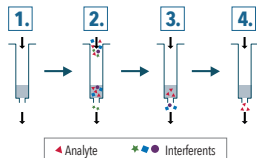


Centrifugation 15000g for 20min
And supernatant recovery

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 μ L 0.1%AF in H₂O
- 4. Elution:**
2 x 500 μ L 80%MeOH 0.1%AF
- 5. Sample evaporation to dryness**

Step 8 Sample injection



Solubilization in **150 μ L** of starting mobile phases

Put it in vial

Injection: **2 μ L**

Step 9 Data preprocessing



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

The screenshot shows the 'Import New Sample' form in the HCPprofiler application. The form includes fields for 'Sample name' (Sample_1_HCPprofiler), 'Fractionnement' (toggle off), 'BEADS diluted into sample' (toggle on), 'Final Sample volume' (150 μ L), and 'Injected volume' (2 μ L). There are also fields for 'Quality control accession - opt' (CG-1), 'Injected QC quantity - opt' (X, fold), 'Drug substance (Accession) - opt' (DS), 'Isoelectric Point - opt' (empty), and 'Injected drug substance quantity - opt' (200 ng). An 'Import Sample' button is at the bottom. A green box highlights the 'Quality control accession' and 'Injected QC quantity' fields, and an orange box highlights the 'Isoelectric Point' field. A green arrow points from the text 'Possibility of adding a quality control before the digestion step' to the green box, and an orange arrow points from the text 'Not necessary with proteome discoverer' to the orange box.

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