

In-solution digest protocol

UCSF Chem219 2010

Reagents:

H₂O: HPLC water
ACN: HPLC grade acetonitrile
trypsin: Promega sequencing grade modified trypsin
Lys-C: Roche Biosciences

Solutions:

ABC= 25 mM ammonium bicarbonate
Urea= 8M Urea in ABC (pH ~8)
DTT= 50 mM dithiothreitol (DTT) in ABC
IAM= 50 mM iodoacetamide in ABC

Denature protein:

Add 10ul freshly-made urea solution to the 10 ul of sample and vortex.

Reduce disulfide bonds:

Add 2 ul DTT solution (to give a total DTT concentration of ≈5 mM) and vortex. Incubate at 60°C for 30 mins.

Alkylate –SH:

Allow solution to cool, then add 6ul IAM solution (to give an IAM concentration of ≈15 mM) and vortex. Incubate in dark at room temperature for 45 mins.

Stop alkylation:

Add 2ul DTT solution. This will react with any remaining IAM.

Split and dilute sample:

Add 10 ul ABC, to give a total volume of 40 ul (and a urea concentration of 2M). Equally split the sample in half for subsequent trypsin and Lys-C digestion.

Digest: (Ask Kathy for trypsin and LysC)

Add trypsin (2μL, 50ng/ μL) or LysC (1 μL, 100ng/ μL. Incubate at 37C overnight.

Stop Digest (Day 2):

Add 10 ul of 10 % formic acid to each sample and vortex. The acidification will stop the digestion and is also required for the reverse-phase clean-up (next step).

Desalting:

See Ziptip protocol.