

Sample Prep — Protein Extraction, Digestion and Cleanup — Hands-on

Go from complex biological samples to LC–MS/MS ready peptide mixtures with confidence. This module focuses on the theory and practical decision-making behind protein extraction, solubilization, digestion and cleanup so that your downstream identification and quantification are not compromised by poor sample quality.

Sample Prep — Protein Extraction, Digestion and Cleanup

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Session 1

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Lysis & Protein Extraction Basics

Overview of sample types and matrix challenges

[cells and tissues](#) [biofluids](#) [membrane rich samples](#)

Lysis and solubilization strategies (conceptual)

[mechanical vs chemical lysis](#) [detergents and chaotropes](#) [protease and phosphatase inhibitors](#)

Protein recovery, clarification and storage considerations

clarification and removal of debris **buffer**
compatibility with LC–MS/MS **aliquoting and**
freeze protection

Session 2

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Reduction, Alkylation & Digestion Chemistry

Disulfide bond management and denaturation choices

why reduction and alkylation **impact on peptide**
mapping **common pitfalls**

Enzymatic digestion strategies (theory level)

trypsin and other proteases **on pellet vs in solution**
logic **missed cleavages and sequence coverage**

Designing digestion workflows for different sample types

low vs high complexity samples **time and**
temperature constraints **compatibility with labeling**
strategies

Session 3

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Cleanup, Fractionation & Enrichment

Why cleanup is essential before LC–MS/MS

removing salts and detergents **improving sensitivity**
and robustness **carryover considerations**

Concepts of solid phase cleanup and fractionation

reversed phase at peptide level **offline fractionation**
logic **orthogonality of separations**

Enrichment strategies for targeted sub proteomes (overview)

depletion of high abundance species **basic PTM and peptide enrichment concepts** **trade offs between depth and throughput**

Session 4

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QC, Troubleshooting & Study Templates

Assessing protein and peptide quality prior to LC–MS/MS

theory plus checklist exercise

Common failure modes and how to reason about them

low protein yield **poor digestion patterns** **carryover and contamination indicators**

Building sample prep sections for a proteomics study plan

template for methods sections **sample mapping sheets** **handoff checklists to MS core**