APPLICATIONS

Lys-C Protease for Improvements in Peptide Mapping Workflows

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Overview

Peptide mapping is a ubiquitous method within protein characterization. The general workflow includes the isolation of a protein, followed by in-solution digest using a serine protease to yield peptides, which are subsequently separated by LC and analyzed by UV and/or MS techniques. Because of its specificity and the general size of peptides generated, trypsin is most commonly used. However, trypsin can have missed cleavages, particularly with lysine. Therefore, a common approach is to supplement the digestion with Lys-C, another serine protease. In this application note, we demonstrate the sequence coverage results for NIST mAb digestion between standard in-solution trypsin when compared to a trypsin/Lys-C digest.

The number of unique peptides yielded from the trypsin-only digestion was 267, when compared to the trypsin/Lys-C digestion with 288 (full peptide maps shown in **Figure 1**). Importantly, the DMIF peptide, as shown in **Figure 2**, is recovered significantly more with the trypsin/Lys-C digestion. This result allowed for the DMIF peptide to be identified by the information-dependent acquisition (IDA) MS/MS experiment. As such, sequence coverage of 91.6% for the heavy chain for the trypsin/Lys-C digestion, whereas trypsin-only yielded an 85.8% sequence coverage.

In summary, peptide mapping workflows should be optimized to improve digestion efficiency and reproducibility, and one strategy is to use the serine protease Lys-C to overcome any missed cleavages or digestion inefficiencies.

Digestion Procedure:

Step	Details
Denaturation	To sample, add 1:1 (v:v) of 5 M Guanidine
Reduction	1:10 (v:v) 200 mM DTT:Protein
	Incubate at 57 °C for 30 min, shaking at 1000 rpm
Alkylation	1:2 (v:v) 400 mM iodoacetamide (IAM): DTT
	Incubate in the dark 45 min Quench, 1:2 (v:v) 200 mM DTT: IAM
Buffer Exchange	100 mM Ammonium Bicarbonate, overnight
Digestion	1:20 (w/w) Trypsin:Sample or 1:20 (w/w) Trypsin/Lys-C:Sample
	Incubate 37 °C for 6 h, shaking at 1000 rpm
Reaction Quench	Formic acid
	SpeedVac to dryness, resuspend in mobile phase prior to analysis

LC Conditions

Column: bioZen™ 2.6 µm Peptide XB-C18
Dimension: 150 x 2.1 mm
Part No.: 00F-4768-AN
Recommended Guard: SecurityGuard™ ULTRA
Guard Cartridge Part No.: AJ0-9806
Guard Holder Part No.: AJ0-9000
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Flow Rate: 0.3 mL/min
Gradient: 1-50% B in 50 minutes
Temperature: 40 °C
Detector: Q-TOF (SCIEX® X500B)
Sample: Digested NIST mAb

Figure 1. Comparison of TICs- Trypsin vs Trypsin/Lys-C

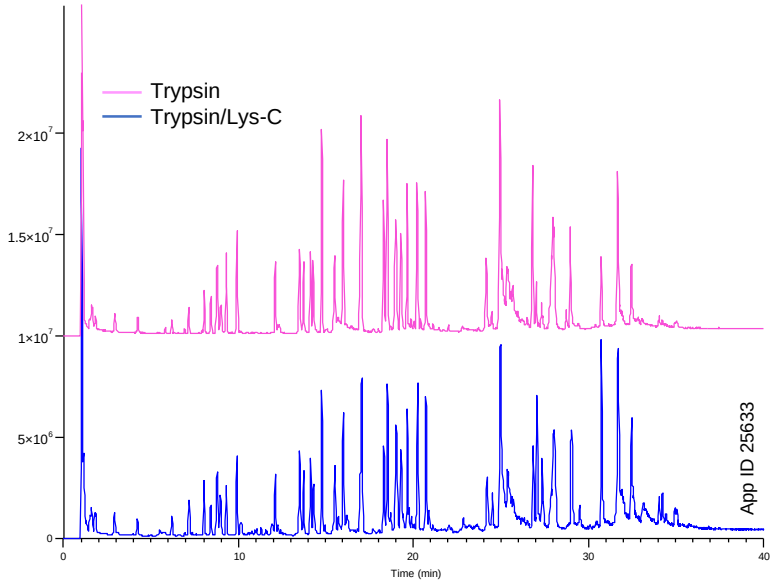
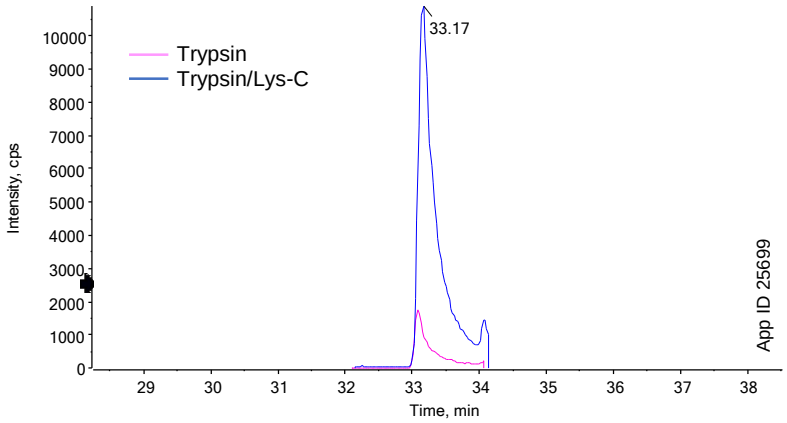


Figure2. XIC Comparison, DMIF Peptide



Comparison of Sequence Coverage

Trypsin/Lys-C Digested NIST mAb

Heavy Chain Sequence Coverage 91.6%

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVG
WIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKD
TSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWG
QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

Trypsin Digested NIST mAb

Heavy Chain Sequence Coverage 85.8%

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVG
WIRQPPGKALEWLADIWWDDKKHYNPSLKDRLTISKD
TSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWG
QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKS
CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPA
PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

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