

APPLICATIONS

Lys-C Protease for Improvements in Peptide Mapping Workflows

M. Christina Malinao and Brian Rivera
 Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501, USA

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

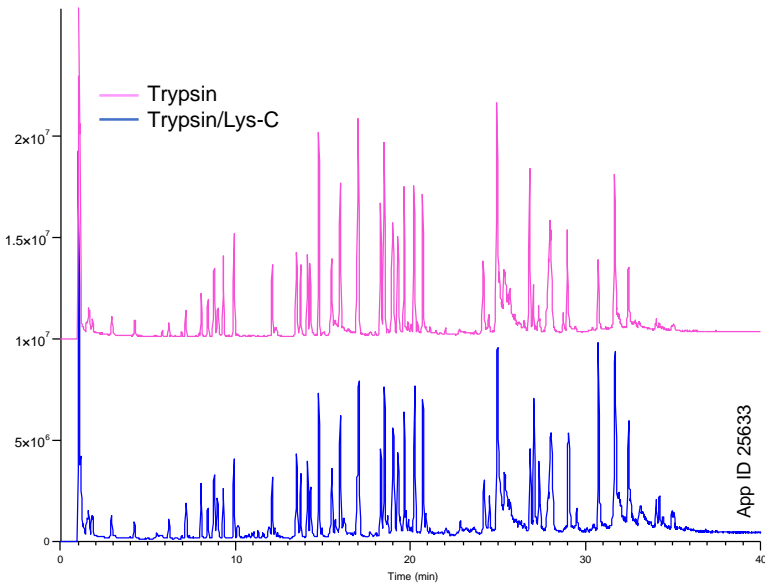
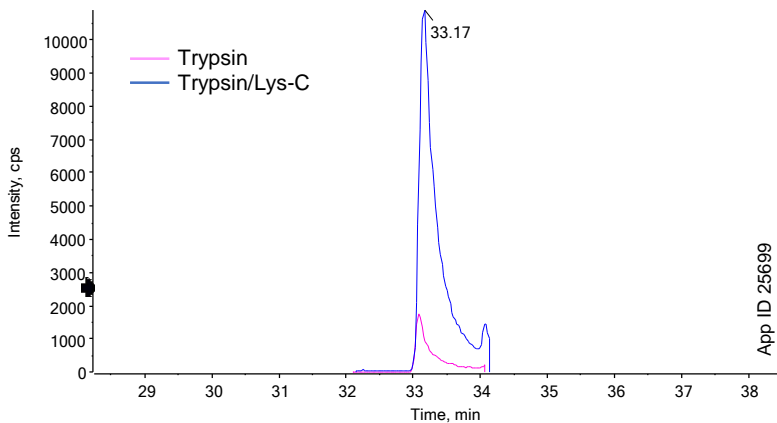


Figure 2. XIC Comparison, DMIF Peptide



Comparison of Sequence Coverage

Trypsin/Lys-C Digested NIST mAb

Heavy Chain Sequence Coverage 91.6%

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVG
 WIRQPPGKALEWLADIWDDKKHYNPSLKDRLLTISKD
 TSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWG
 QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDRVEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT
 PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
 EQY**N**STYR**V**VS**V**LT**V**LV**H**Q**D**W**L**NG**K**E**Y**K**C**K**V**S**N**K**A**L**P**A
 P**I**E**K**T**I**S**K**A**K**G**Q**P**R**E**P**Q**V**Y**T**L**P**P**S**R**E**E**M**T**K**N**Q**V**S**L**T**C
 L**V**K**G**F**Y**P**S**D**I**A**V**E**W**E**S**N**G**Q**P**E**N**N**Y**K**T**T**P**P**V**L**D**S**D**G**S**F
 F**L**Y**S**K**L**T**V**D**K**S**R**W**Q**Q**G**N**V**F**S**C**S**V**M**H**E**A**L**H**N**H**Y**T**Q**K**S**L
 S**L**S**P**G**K**

Trypsin Digested NIST mAb

Heavy Chain Sequence Coverage 85.8%

QVTLRESGPALVKPTQTLTLTCTFSGFSLSTAGMSVG
 WIRQPPGKALEWLADIWDDKKHYNPSLKDRLLTISKD
 TSKNQVVLKVTNMDPADTATYYCARDMIFNFYFDVWG
 QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCL
 VKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
 SSVVTVPSSSLGTQTYICNVNHKPSNTKVDRVEPKS
 CDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRT
 PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE
 EQY**N**STYR**V**VS**V**LT**V**LV**H**Q**D**W**L**NG**K**E**Y**K**C**K**V**S**N**K**A**L**P**A
 P**I**E**K**T**I**S**K**A**K**G**Q**P**R**E**P**Q**V**Y**T**L**P**P**S**R**E**E**M**T**K**N**Q**V**S**L**T**C
 L**V**K**G**F**Y**P**S**D**I**A**V**E**W**E**S**N**G**Q**P**E**N**N**Y**K**T**T**P**P**V**L**D**S**D**G**S**F
 F**L**Y**S**K**L**T**V**D**K**S**R**W**Q**Q**G**N**V**F**S**C**S**V**M**H**E**A**L**H**N**H**Y**T**Q**K**S**L
 S**L**S**P**G**K**

APPLICATIONS

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/LiveChat.

Australia

t: +61 (0)2-9428-6444
auiinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
info@phenomenex.com

China

t: +86 400-606-8099
cninfo@phenomenex.com

Denmark

t: +45 4824 8048
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
eirinfo@phenomenex.com

Italy

t: +39 051 6327511
italiainfo@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
nordicinfo@phenomenex.com

Portugal

t: +351 221 450 488
ptinfo@phenomenex.com

Singapore

t: +65 800-852-3944
sginfo@phenomenex.com

Spain

t: +34 91-413-8613
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
nordicinfo@phenomenex.com

Switzerland

t: +41 (0)61 692 20 20
swissinfo@phenomenex.com

Taiwan

t: +886 (0) 0801-49-1246
twinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
info@phenomenex.com

All other countries Corporate Office USA

t: +1 (310) 212-0555
info@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com



Your happiness is our mission. Take 45 days to try our products. If you are not happy, we'll make it right.
www.phenomenex.com/behappy

Terms and Conditions

Subject to Phenomenex Standard Terms and Conditions, which may be viewed at www.phenomenex.com/TermsAndConditions.

Trademarks

bioZen, SecurityGuard, and Be-Happy are trademarks of Phenomenex. Sciex is a registered trademark of AB Sciex Pte. Ltd. AB SCIESM is being used under license.
FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2020 Phenomenex, Inc. All rights reserved.