APPLICATIONS

Comparison of Trypsin Digestion pH for Peptide Mapping Workflows

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Overview

Peptide mapping is a common method for 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. Trypsin has an optimal activity at around pH 8. However, under even moderately basic conditions, deamidation can occur. In this application note, we investigate the differences in digestion between a standard ammonium bicarbonate at pH 8.2 and a 1X PBS, pH 7.4.

As we can see in **Figure 1**, there is a clear difference visually when looking at TICs for the standard digestion in ammonium bicarbonate when compared to PBS. Appreciable peaks before 15 minutes are not observed when using PBS, indicating the absence of smaller tryptic peptides. This result may indicate missed cleavages due to the suboptimal pH by which the digestion is occurring.

However, in observing peptides that may be prone to deamidation, namely the "VVSV" peptide, we can see that there is significantly more deamidated peptides in the ammonium bicarbonate buffers. The two later eluting peaks indicate the deamidated VVSV peptides, with the bottom trace indicating significantly higher relative peak heights (**Figure 2**).

One interesting note is that sequence coverage between both samples prepared was relatively similar (91.7% for bicarbonate, 86.6% for PBS). Missed cleavages do not necessarily mean indicate a decrease in sequence coverage, as longer peptides may still be identified by tandem MS. However, missed cleavages may introduce variability in the digestion which should be assessed depending on the scope of the method.

In summary, there may be some variation when digestion is performed at suboptimal pH. Therefore, it is prudent to investigate the trypsin digestion pH to assess the impact on a particular protein of interest.

Digestion Procedure:

Step	Details
Denaturation	To sample, add 1:1 (v:v) 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 or 1X Phosphate Buffered Saline, pH 7.4, overnight
Digestion	1:20 (w/w) Trypsin: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: Tryptic digest, NIST mAb

Figure 1. Comparison of TICs, pH 8.2 vs pH 7.4

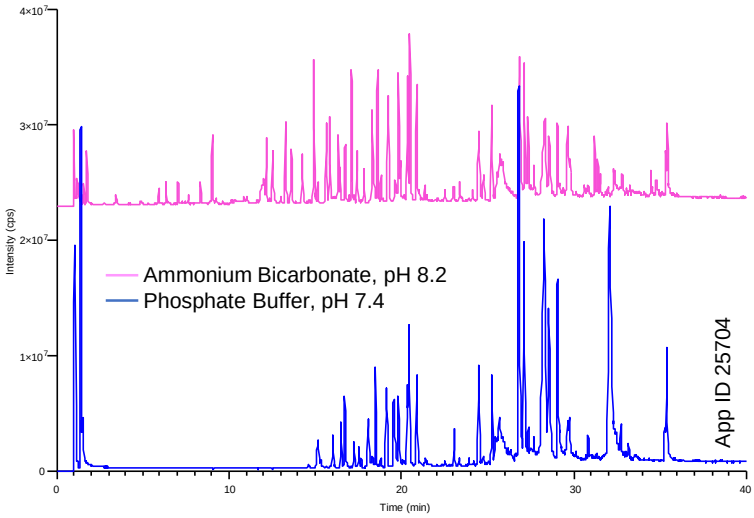
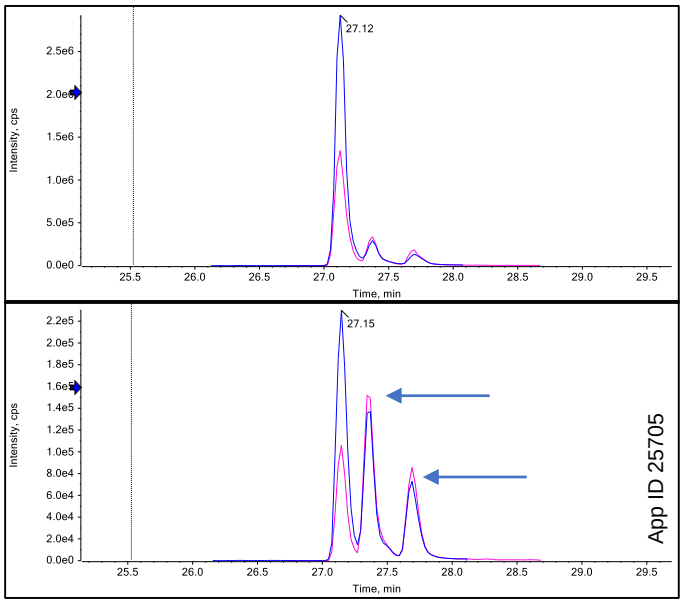


Figure 2. XIC Comparison, VVSV Peptide



Comparison of Sequence Coverage

Ammonium Bicarbonate Digested Trastuzumab

Heavy Chain Sequence Coverage 91.7%

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIH
WVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTI
SADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFY
AMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Phosphate Buffer (PBS) Digested Trastuzumab

Heavy Chain Sequence Coverage 86.6%

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIH
WVRQAPGKGLEWVARIYPTNGYTRYADSVKGRFTI
SADTSKNTAYLQMNSLRAEDTAVYYCSRWGGDGFY
AMDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA
VLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPS
NTKVDKKVEPPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQ
DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP
QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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