SIMPLE AND AUTOMATABLE SAMPLE PREPARATION FOR TRYPTIC PEPTIDE MAPPING WITH THE PEPTIDEWORKS KIT

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INTRODUCTION

Reliable day-to-day sample preparation is a critical factor in generating an effective peptide mapping method. Sample preparation for peptide mapping can result in method-induced peptide modifications, over- or under-digestion of the protein, and autolysis of the proteolytic enzyme, each of which complicate data analysis and interpretation.

Waters' PeptideWorks™ Tryptic Protein Digestion Kits deliver fast and reliable sample preparation for routine peptide mapping of therapeutic proteins. The sample preparation kit is centered around RapiZyme™ Trypsin, Waters' homogeneously methylated, recombinant trypsin. RapiZyme Trypsin enables speed, digestion fidelity, and low levels of trypsin-derived peptides through its autolysis resistance, purity, and high activity. RapiZyme Trypsin can be used at high concentrations to achieve faster digestions without requiring high temperatures or sacrificing digestion completeness.

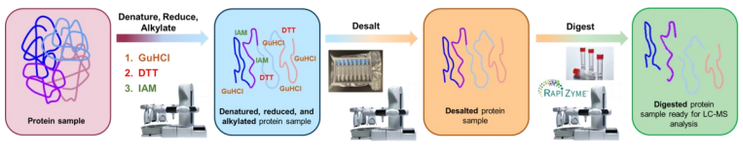


Figure 1. Workflow for the preparation of tryptic protein digests using the PeptideWorks Tryptic Protein Digestion Kit. The protocol can be performed manually or with automation on the Andrew+™ Pipetting Robot .

WORKFLOW & AUTOMATION

The PeptideWorks Kit workflow was optimized to enable fast and complete digestion of proteins by RapiZyme Trypsin. The workflow can be performed manually or with automation on the Andrew+ Pipetting Robot .

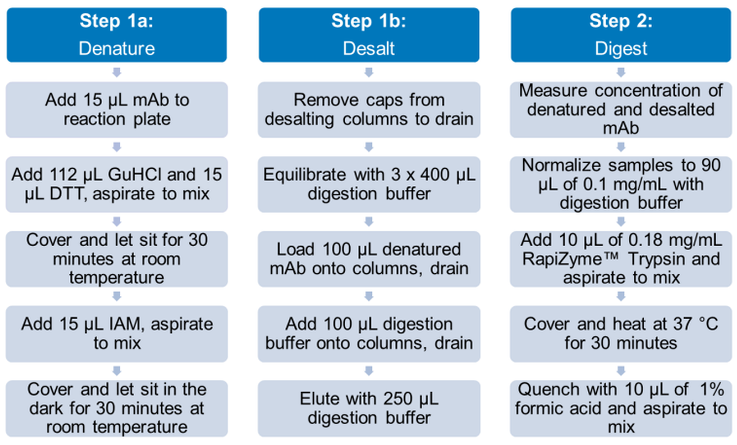
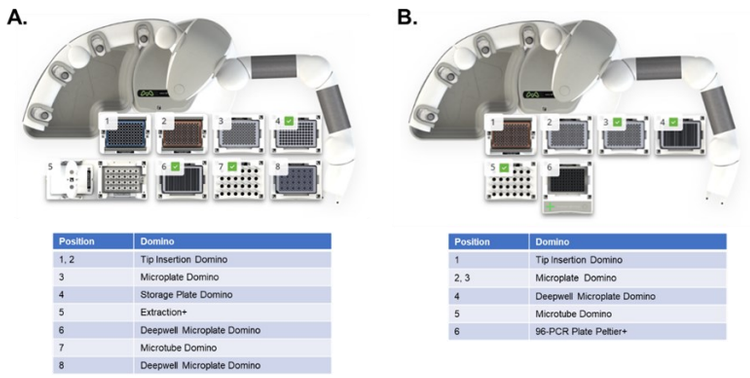


Figure 2. Flow diagram outlining the PeptideWorks Kit sample preparation workflow. Both manual and automated sample preparation follow the depicted workflow.



Position	Domino
1, 2	Tip Insertion Domino
3	Microplate Domino
4	Storage Plate Domino
5	Extraction+
6	Deepwell Microplate Domino
7	Microtube Domino
8	Deepwell Microplate Domino

Position	Domino
1	Tip Insertion Domino
2, 3	Microplate Domino
4	Deepwell Microplate Domino
5	Microtube Domino
6	96-PCR Plate Peltier+

Figure 3. Andrew+ Robot deck layouts for the 24-sample automated workflow. Protocol A executes denaturation, reduction, and alkylation and protocol B executes concentration normalization and digestion.

DIGESTION COMPLETION

Both manual and automated sample preparation yield comparable chromatographic results with high sequence coverage (>88% expected peptides). Both workflows deliver NISTmAb digests with less than 5% missed and non-specific cleavages, indicating high digestion efficiency without over-digestion of the protein.

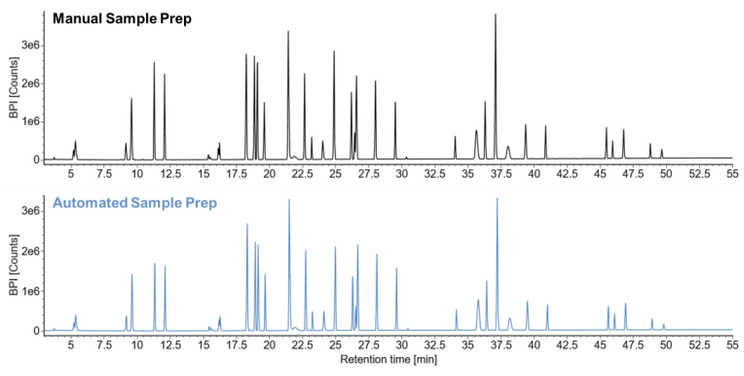


Figure 4. BPI chromatograms of NISTmAb digests prepared using the PeptideWorks Kit. Manual and automated workflows show equivalent results.

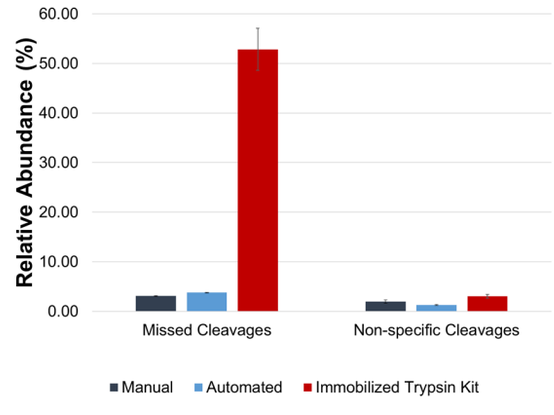
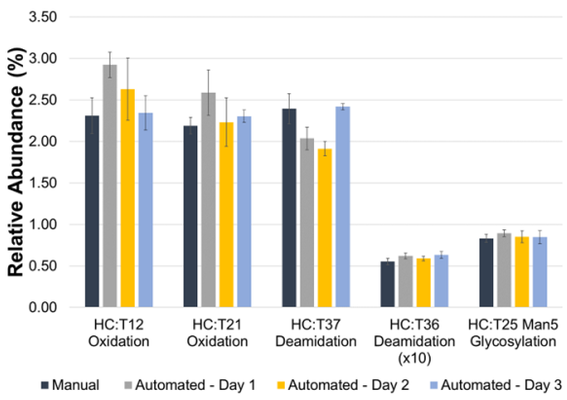


Figure 5. Relative abundance of missed and non-specific cleavages for NISTmAb digests prepared using the PeptideWorks Kit and a leading immobilized trypsin kit. Error bars represent one standard deviation.

PEPTIDE MODIFICATIONS

The PeptideWorks Kit enables fast preparation of tryptic peptide mapping samples without sacrificing digestion completion or inducing high levels of method-induced deamidation or oxidation. The day-to-day variability of the unmodified peptide abundance was less than 15% for each peptide, demonstrating consistent peptide recovery and therefore consistent quantification limits. Day-to-day variability of the modified peptide relative abundance was less than 10% for each peptide modification.



Peptide (Modification)	% RSD	
	Unmodified Peptide Abundance	Modified Peptide Relative Abundance
HC:T12 (Ox.)	11.80	8.06
HC:T21 (Ox.)	6.02	6.79
HC:T37 (Deam.)	8.09	8.82
HC:T36 (Deam.)	4.62	3.01
HC:T25 (Man5)	6.47[a]	2.12

[a] The combined abundance of the G0F, G1F, and G2F glycoforms of HC:T25 were used in place of the unmodified peptide abundance.

Figure 6. (TOP) Bar chart representing the relative abundance of select peptide modifications for NISTmAb digests prepared using the PeptideWorks Kit. Error bars represent one standard deviation. (BOTTOM) Table outlining the % RSD of the unmodified peptide abundance and modified peptide relative abundance.

ADC PEPTIDE MAPPING

The PeptideWorks Kit was used for peptide mapping of a Trastuzumab biosimilar (T mAb) and Trastuzumab emtansine (T-DM1), a lysine-linked ADC. Additional peaks are observed in the more retained region of the T-DM1 chromatogram due to presence of the conjugated drug. Some diastereomer peptide pairs, which result from two possible stereochemical configurations during the conjugation reaction, were identified in the T-DM1 chromatogram using MS data.

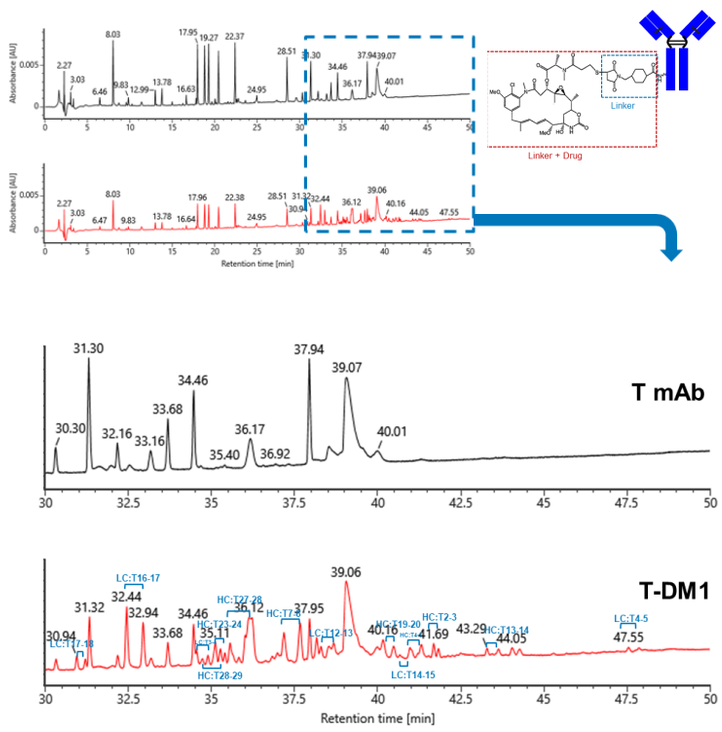


Figure 7. UV chromatograms of tryptic digests of a TrastuzumAb biosimilar (T mAb) and Trastuzumab emtansine (T-DM1) prepared using the PeptideWorks Kit.

CONCLUSION

- **Comprehensive kit and protocol for preparation of tryptic protein digests**
- **Automatable preparation of 24 samples in under 2.5 hours**
- **Reproducible sample prep without sacrificing digestion completion or generating high levels of method-induced peptide modifications**
- **93% reduction in missed cleavages and 55% reduction in non-specific cleavages compared to a leading immobilized trypsin digest kit**
- **Suitable for peptide mapping of ADCs**

METHODS

Samples: NISTmAb (RM 8671), Trastuzumab-anns, Trastuzumab emtansine

Instruments: ACQUITY™ UPLC™ I-Class PLUS System, ACQUITY RDa™ Detector (NISTmAb work), Xevo™ G2 XS Mass Spectrometer (ADC work)

Columns: ACQUITY Premier Peptide CSH™ C18 Column, 1.7 µm, 2.1 x 150 mm (NISTmAb work), XSelect™ Premier Peptide CSH C18 Column, 2.5 µm, 2.1 x 150 mm (ADC work)

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