Rapid Quantitation of IgG after Digestion at Elevated Temperature with a Novel Trypsin Reagent

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1. Introduction

As increasing numbers of protein-based therapeutics enter development pipelines, more efficient protocols are required for structural characterization and quantitation. Proteolysis of these proteins into peptides represents a bottleneck and often requires optimization of numerous steps including reduction, alkylation and digestion time. We have developed a new Trypsin reagent, Rapid Trypsin and Rapid Trypsin with Lys C, that streamlines the entire sample preparation process to less than 1 hour. With this new process, proteolysis is performed at 70°C which facilitates both denaturation and rapid digestion. This protocol is robust, amenable to multiple analytes including pure proteins and complex mixtures, and is compatible with digestion under native, reduced and/or denaturing conditions.

2. Rapid Trypsin Digestion Workflow

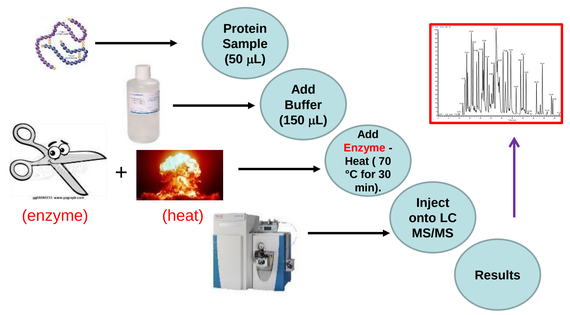


Figure 1. The Rapid Trypsin Digestion workflow is extremely simple as well as flexible. In many cases, reduction and alkylation are not needed. Direct Proteolysis facilitates MS samples in less than 30 minutes.

3. Efficient Digestion in 30 minutes

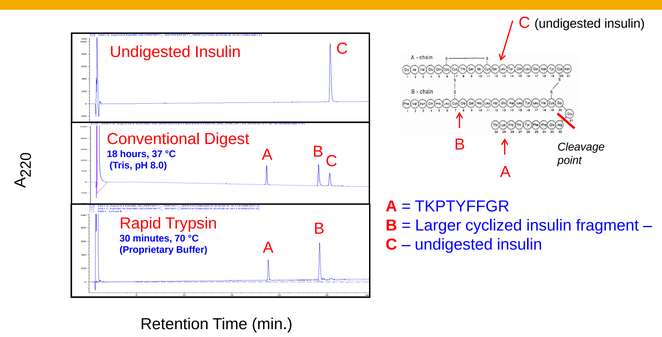


Figure 2. Overnight digestion with traditional buffering reagents, at 37 °C, results in incomplete digestion. The Rapid Trypsin reagent digests insulin within 30 minutes.

4. Time Course of Peptide Formation

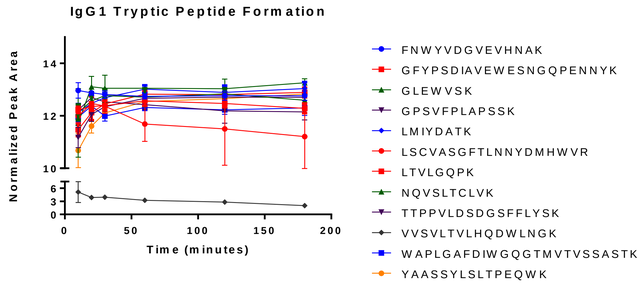
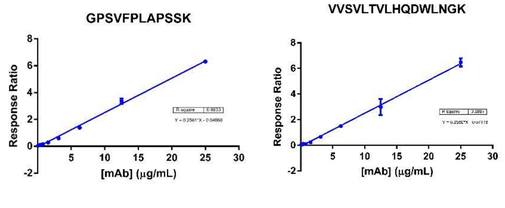


Figure 3. The majority of peptides appear to form within, at most within 20 minutes. A subset require 1 hour. one peptide appears to digest rapidly, but shows some slight degradation due to deamidation. However, this peptides peak area shows very tight precision with respect to peak area.

5. Compatible with Quantitation



QC	[mAb] (µg/mL	% CV	Accuracy
High	5	5.86	87.48
Medium	0.5	13.11	92.8
Low	0.1	1.23	111.4

QC	[mAb] (µg/mL	% CV	Accuracy
High	5	3.38	86.7
Medium	0.5	8.07	103.1
Low	0.1	5.62	95.1

N=3 replicates for Calibration curve; N=4 for QC

Figure 4. The Rapid Trypsin protocol can be used for quantitative analytical applications. Both precision and accuracy are within GLP guidelines with strong linearity from 0.1 – 25 µg/mL. All samples included a heavy labeled IgG1 at concentration of 5 µg/mL as an internal standard. All data were processed with Skyline (U. Washington)

6. Rapid Trypsin Yields Precise Data

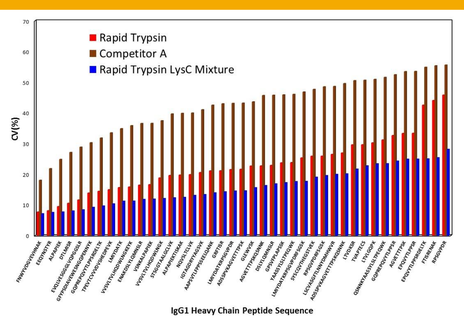


Figure 5. Both Rapid Trypsin and Rapid Trypsin with Lys C give excellent precision, compared to other products with which show higher variability (no peptides are less than 20 % CV). More than 25 IgG1 peptides have CV's less than 20 % when using the Rapid Trypsin reagent

7. Compatible with Reduction and Alkylation

IgG1 Heavy Chain (N=8 replicates)

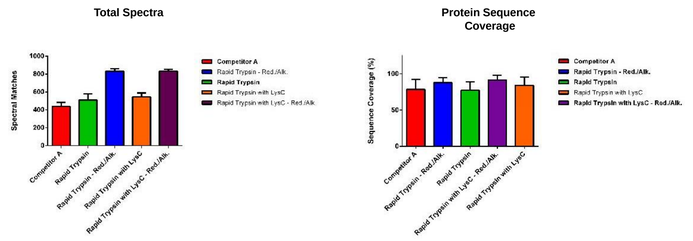


Figure 6. Reduction/alkylation leads to tighter precision, larger number of spectra as well as near complete sequence coverage. Unlike competitive products, Rapid Trypsin is highly flexible as evidenced by it's compatibility with Reduction and Alkylation.

8. The Rapid Trypsin kit is Compatible with Samples after Affinity Enrichment

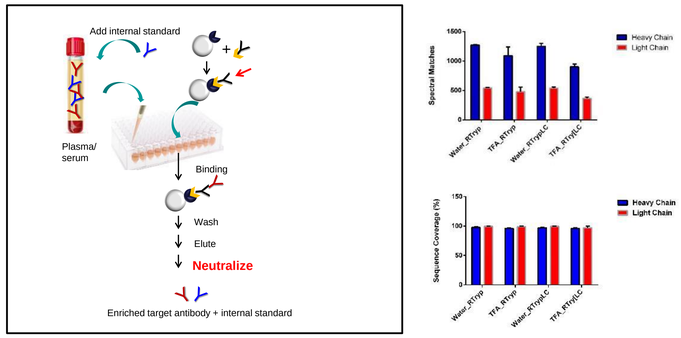


Figure 7. The Rapid Trypsin digestion buffer can replace a neutralization step making the digestion process streamlined, efficient, and rapid. Data on the right indicates the process is robust toward multiple sample type.

9. Conclusions

- Rapid Trypsin reagent can produce digested peptides in as little as 10 minutes.
- Preparation of samples for quantitative analysis yields results with precision and accuracy as good, or better, than those prepared using overnight protocols.
- Workflow is highly flexible and compatible with reduction and alkylation as well as samples prepared using affinity enrichment.
- For optimal sample to sample precision, the Rapid Trypsin/Lys C mixture appears to offer an advantage.

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