

Beyond Trypsin: Choosing the Right Protease for Your Mass Spec Workflow

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Promega GmbH



Promega's Proteomics Portfolio

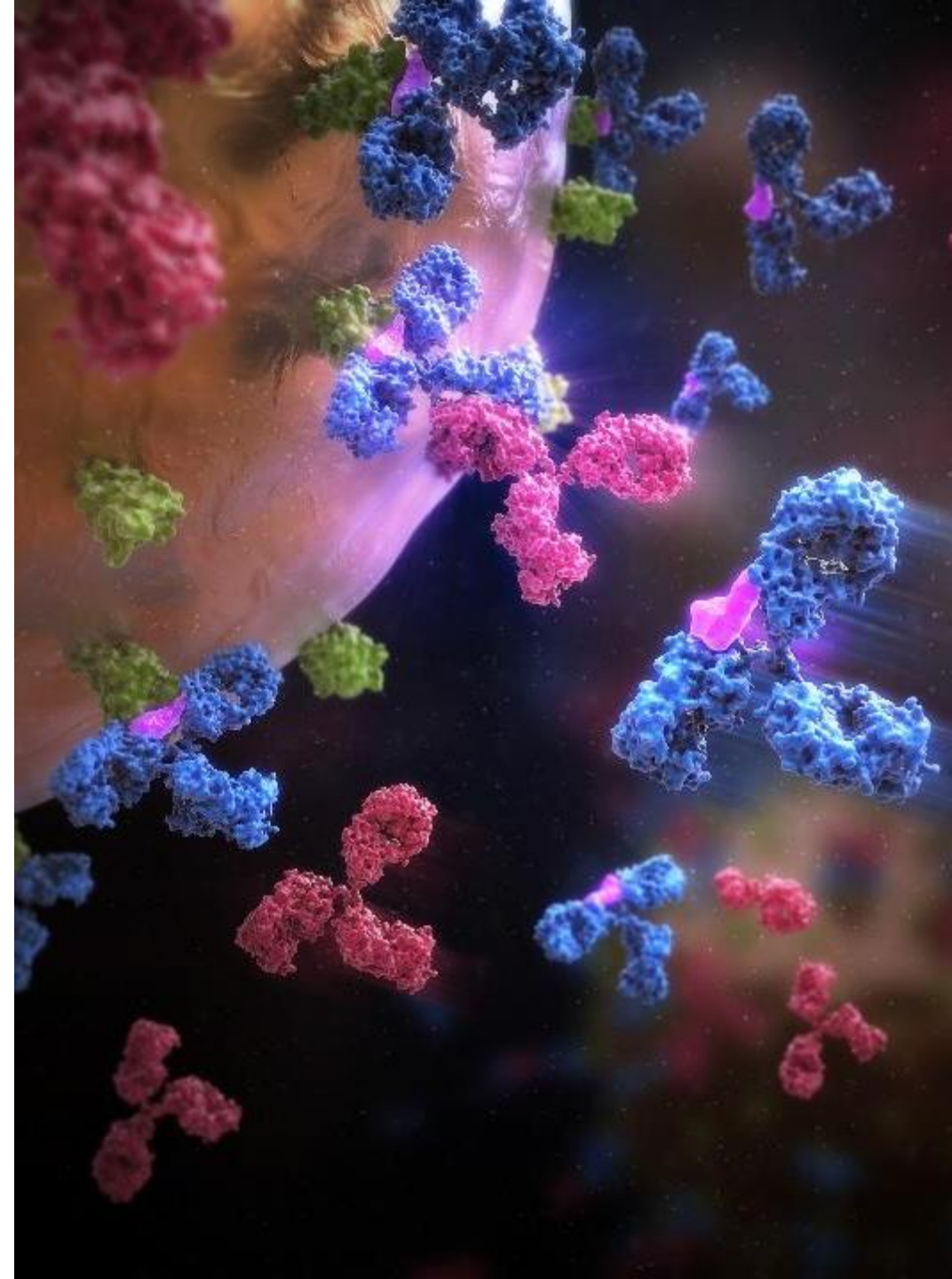
In vitro transcription

Protein expression

Protein purification

Protein detection

MS preparation

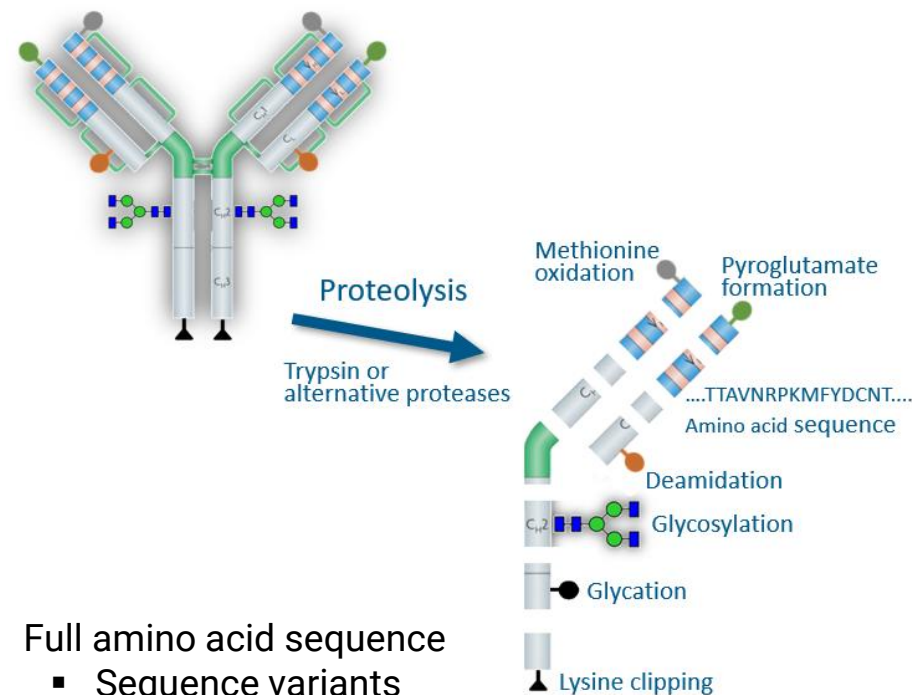
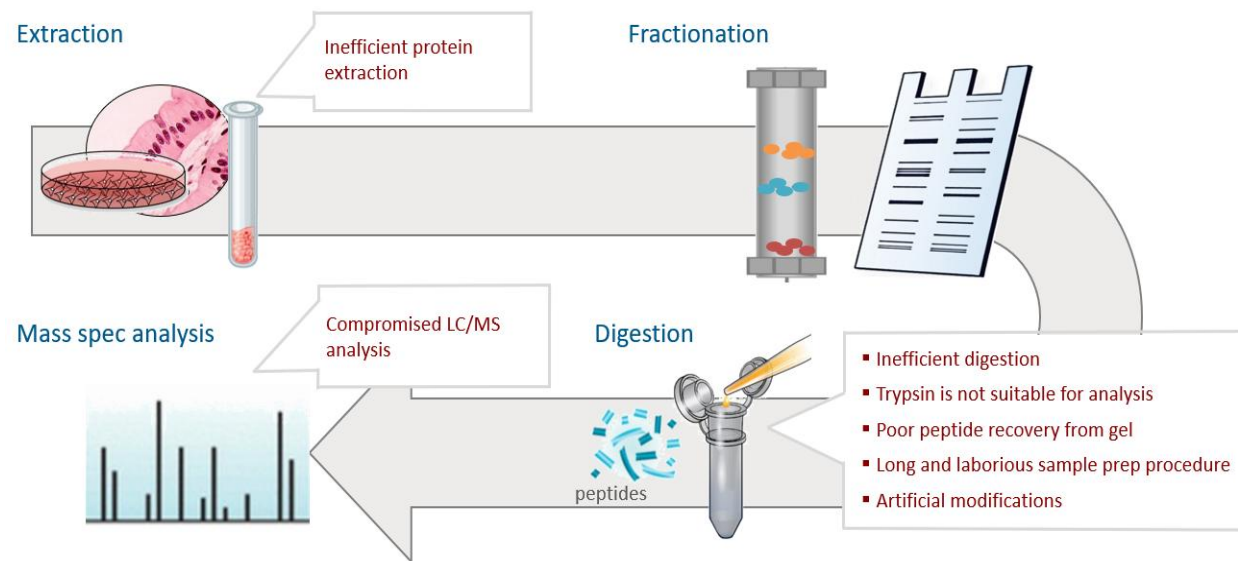


Why MS matters?

- **Quantification**
- **Molecular Weight Determination**
- **Structural Identification**

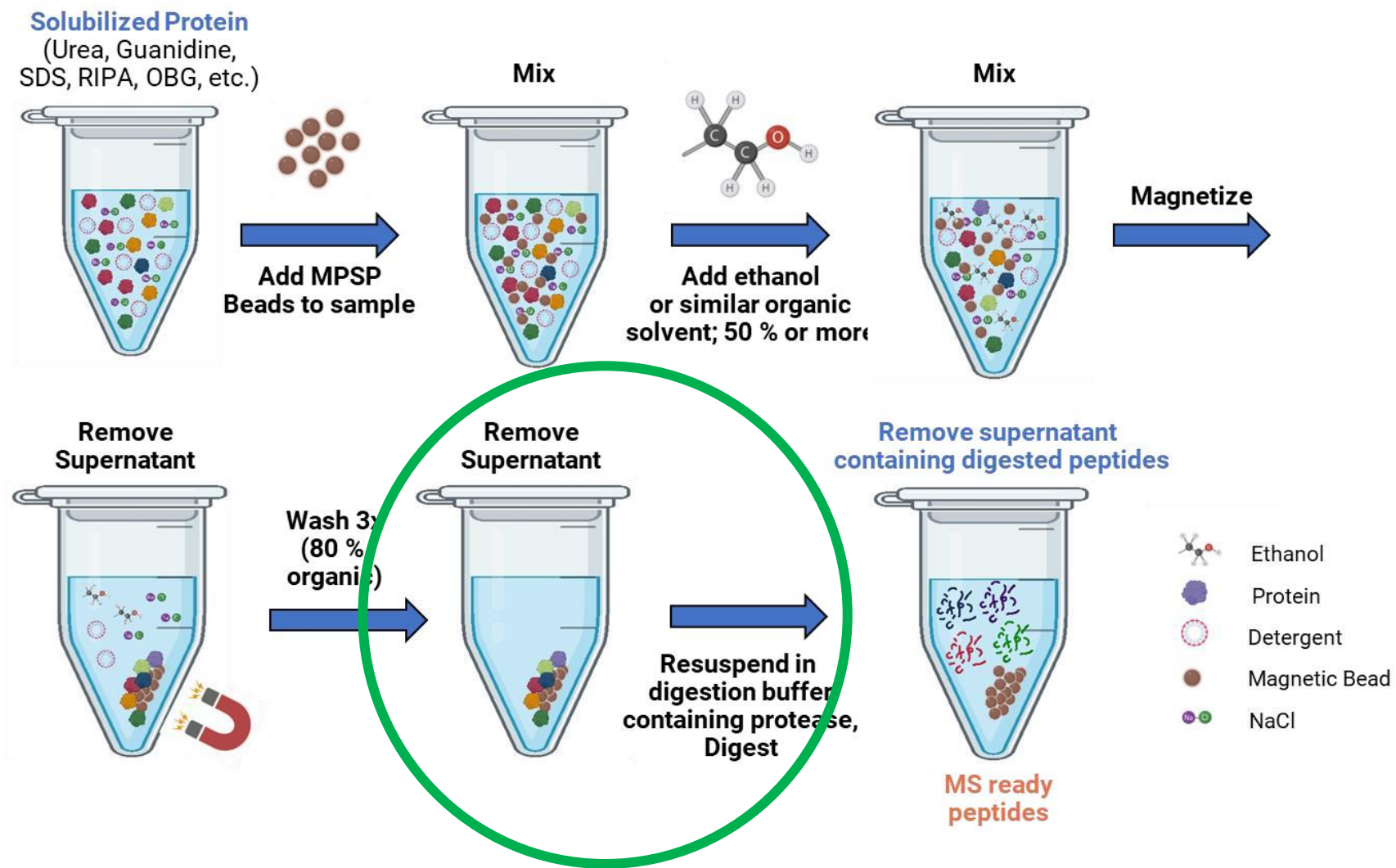
- **High sensitivity**
- **Wide application versatility**
- **Ability to analyze a huge amount of proteins and protein modifications in one experiment**

Protein Sample Prep: Complexity Across Applications



- Full amino acid sequence
 - Sequence variants
- Glycan analysis
- Disulfide bond mapping
- Lysine clipping
- Pyroglutamation, etc.
- Non-enzymatic PTMs

Streamlined Digestion: Magnetic Bead Workflow



Promega's Protein Mass Spec Sample Preparation Portfolio

Trypsin's

- Trypsin, Sequencing Grade
- Trypsin Gold
- Trypsin/Lys-C Mix
- Rapid Trypsin/Rapid Trypsin/Lys-C
- Trypsin Platinum

Alternative Proteases (specific)

- rChymoSelect
- Arg-C Ultra
- Arg-C
- ProAlanase
- rLys-C
- Lys-C
- Glu-C
- rAsp-N
- Asp-N

Reagents for Biotherapeutic protein characterization

- AccuMAP™ low pH digestion kit
- IsoQuant® Isoaspartate Detection Kit
- IdeS and IdeZ
- Endo H
- PNGase F

MS compatible surfactants

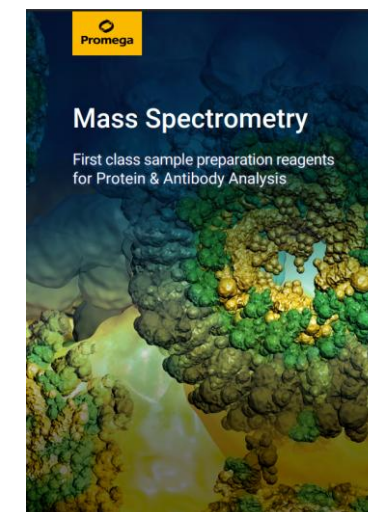
- ProteaseMAX™ (for *in-gel* & *in-solution* digestion)

Protein Reference Materials

- MS compatible yeast and human protein extracts (intact or digest)
- 6 x 5 Peptide Reference Mixture

Alternative Proteases (low- & non-specific)

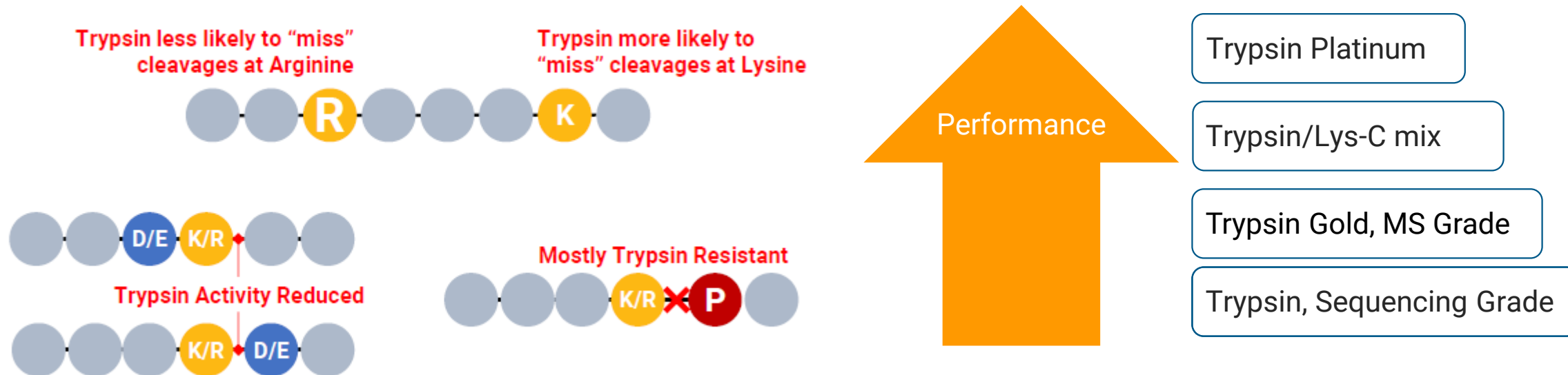
- Chymotrypsin
- Elastase
- Pepsin



Trypsins and its variants



Promega's Trypsins



Trypsin digestion kits for specialized applications

Rapid Trypsin

Rapid and streamlined digestion

AccuMAP™ low pH protein digestion kit

Artificial PTM-free peptide mapping

Trypsin sequencing grade

Reductively methylated
lysine groups to minimize
autolytic digestion

Manufactured by
Promega since 1989!

TPCK treated to inhibit
chymotrypsin activity

Affinity purified to
remove additional
contaminants



Over 17500 Hits on
Google Scholar



[View Google Scholar hits
for “Modified Trypsin”
since 2024](#)

Trypsin Gold, Mass Spectrometry Grade

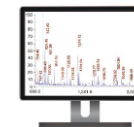
Reductively methylated
lysine groups to minimize
autolytic digestion

Affinity purified to
remove additional
contaminants

TPCK treated to inhibit
chymotrypsin activity



Mass Spec
Qualified



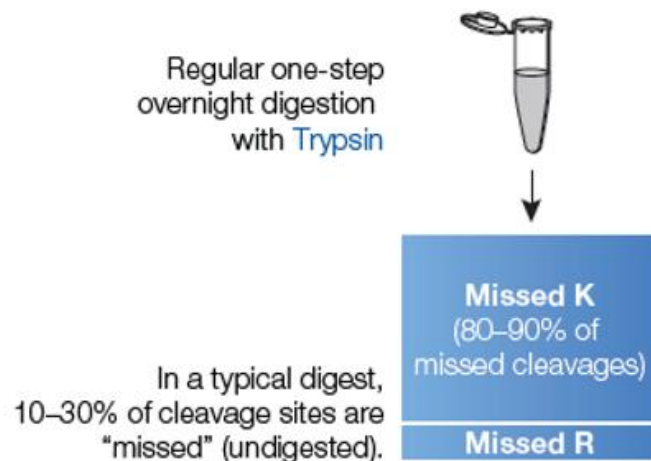
Over 7000 Hits on Google
Scholar



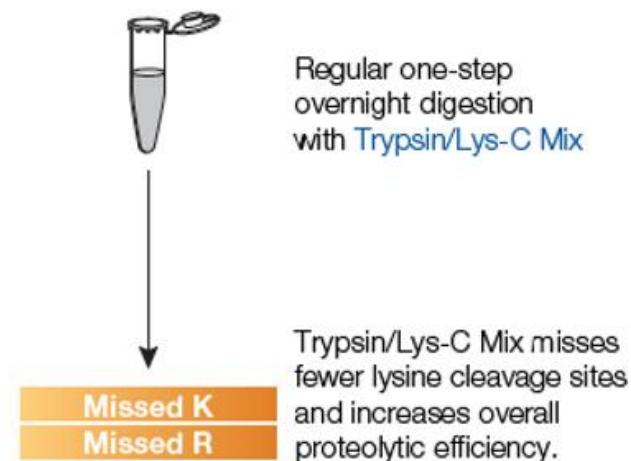
[View Google
Scholar hits for
“Trypsin Gold”
since 2024](#)

Trypsin/Lys-C Mix, Mass Spec grade

Mixture of Trypsin Gold and rLys-C



Digested sample	Missed K	Missed R
Yeast extract	18.6%	3.6%
Mouse extract	6.6%	1.1%

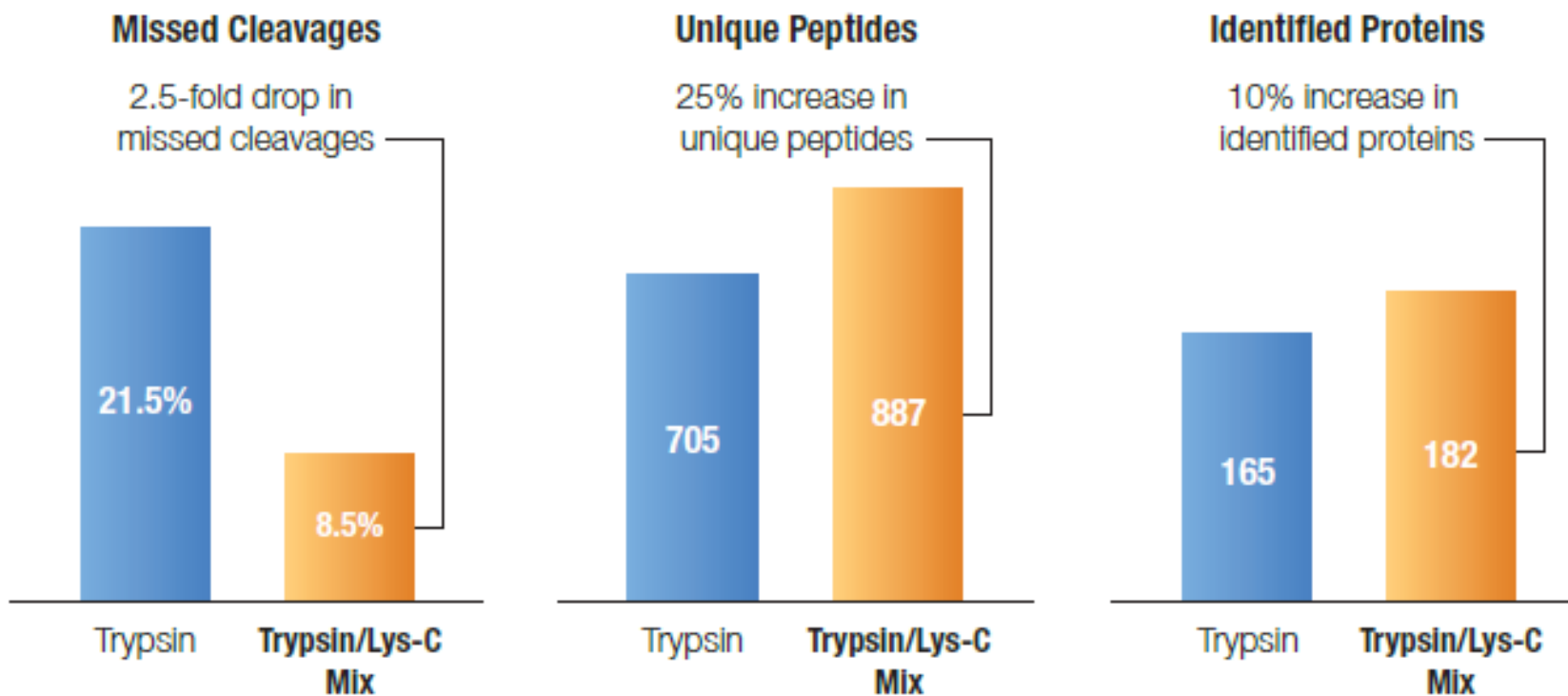


Digested sample	Missed K	Missed R
Yeast extract	2.6%	4%
Mouse extract	2.9%	1.5%

11788 MC

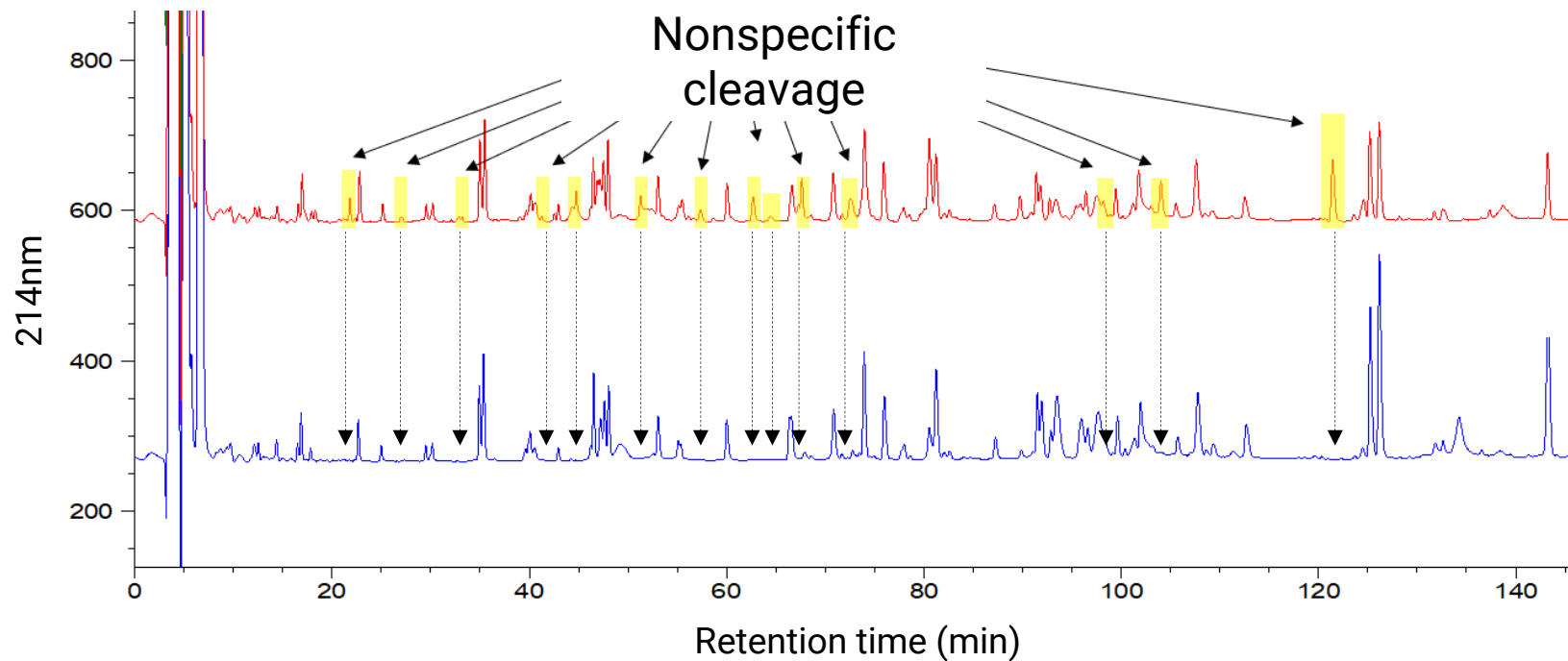
Trypsin/Lys-C Mix, Mass Spec grade

Tolerant for Trypsin-inhibiting contaminants (e.g. organic solvents, denaturants etc.)



Trypsin Platinum, Mass Spec grade

**Recombinant
Enzyme**
Improved purity.
No chymotrypsin
to inhibit!



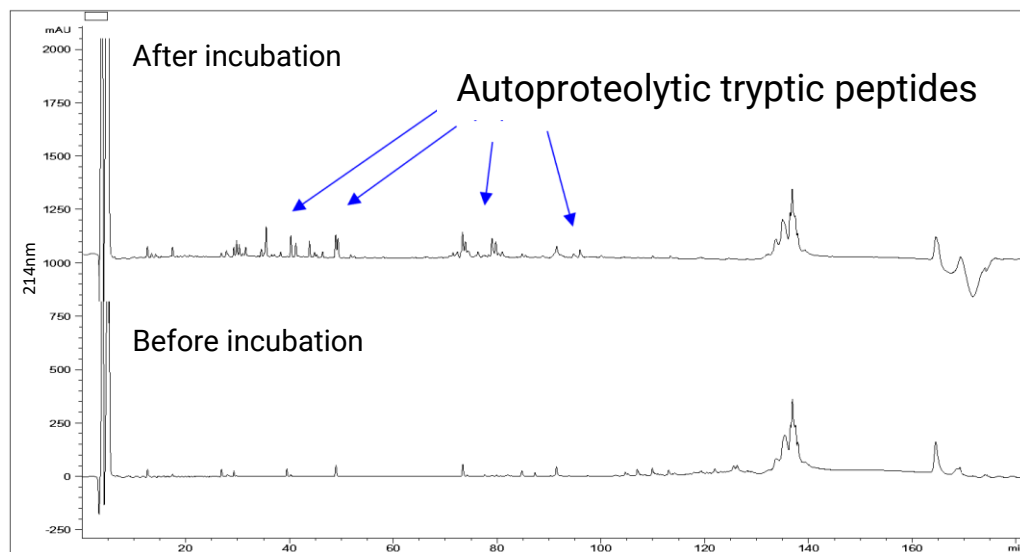
← Digestion with a
commercially available MS
grade trypsin

← Digestion with Trypsin
Platinum

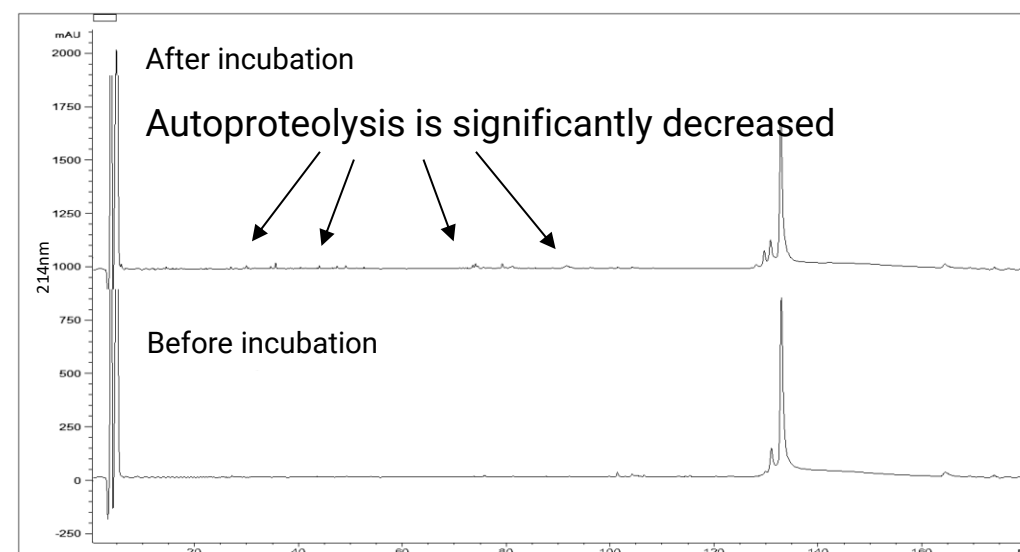
Trypsin Platinum, Mass Spec grade

High resistance to autoproteolysis – robust proteolytic efficiency

Commercially available MS grade trypsin



Trypsin Platinum



→ Trypsin Platinum is free of pre-existing autoproteolytic peptides

Trypsin Platinum, Mass Spec grade

Free of nonspecific cleavage activity

Superior autoproteolytic resistance

Enhanced proteolytic efficiency

Excellent lot-to-lot reproducibility

Free of contaminating animal proteins



[View Google Scholar hits for
"Trypsin Platinum" since 2024](#)

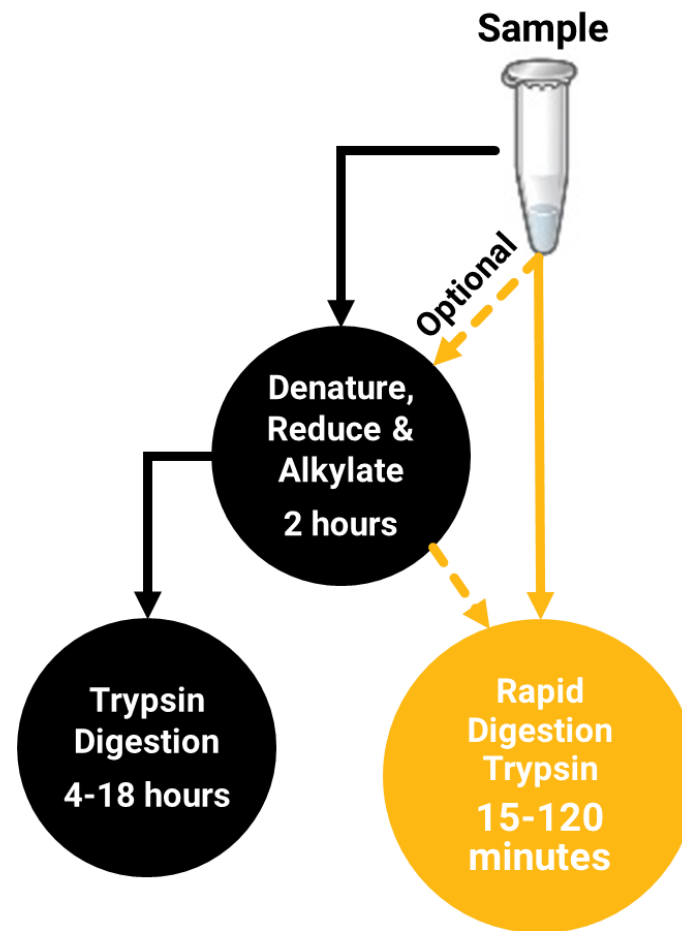
Rapid Trypsin and Rapid Trypsin/Lys-C

Thermostable trypsin, digestion at 70°C

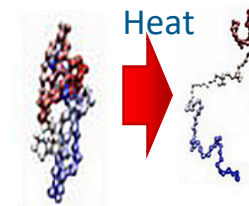
No denaturants, reduction, or alkylation needed

No off-line clean-up prior to MS analysis

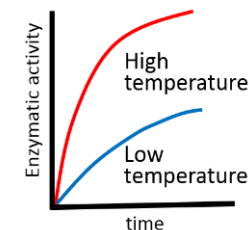
Trypsin/Lys-C format available



Heat advantage



Heat-induced unfolding provides ready protease access to cleavage sites



Heat increases enzymatic activity

Comparison table

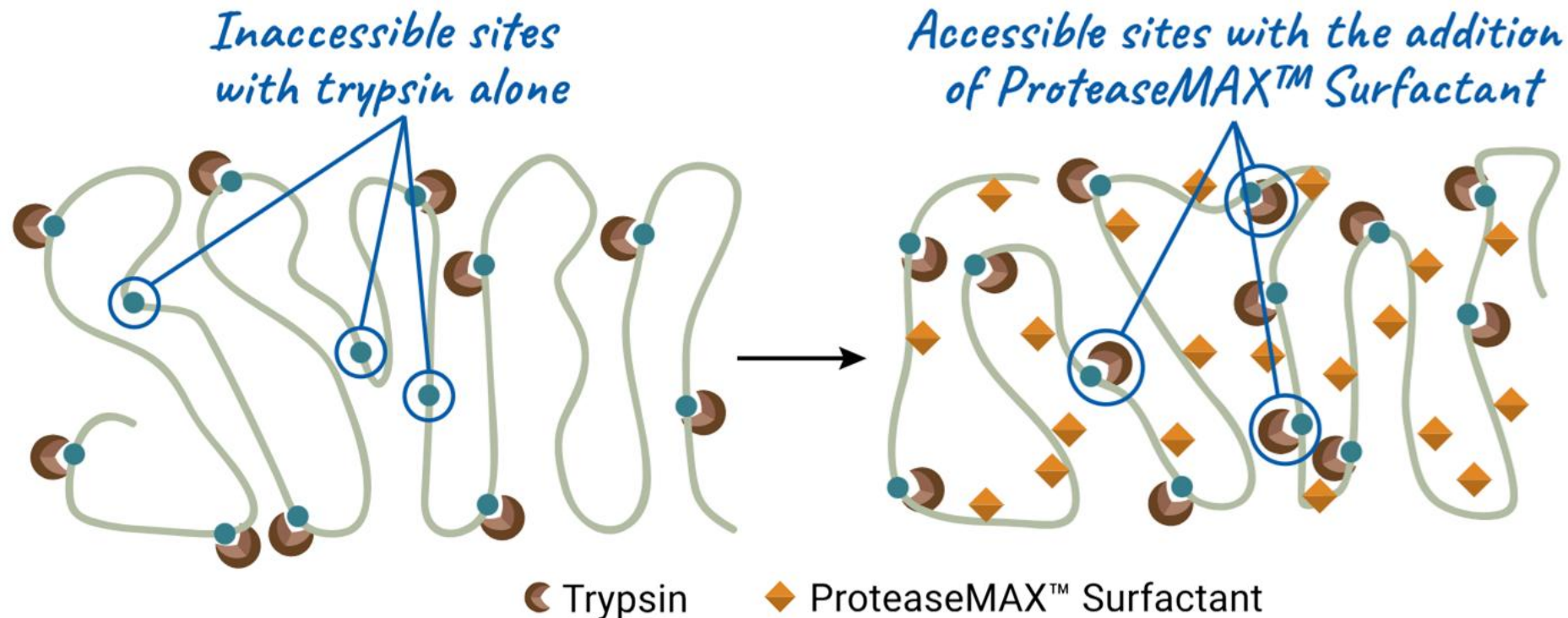
	Trypsin Platinum	Trypsin Gold	Sequencing Grade Trypsin	Trypsin/ Lys-C Mix	Rapid Trypsin	Rapid Trypsin/Lys-C	AccuMAP™ Low pH Kit
Cleavage Specificity	★	✓	✓	✓	✓	✓	✓
Digestion Efficiency	✓	✓	✓	★	✓	★	✓
Autoproteolytic Resistance	★	✓	✓	✓	✓	✓	✓
Purity	★	★	✓	✓	✓	✓	✓
Digestion Speed	✓	✓	✓	✓	★	★	✓
Suppression of Artificial PTMs	✓	✓	✓	✓	✓	✓	★
Tolerance to inhibiting agents	✓	✓	✓	★	✓	★	✓
Customization	★	★	★	★	★	★	★
Highlights	Recombinant enzyme. Higher purity and specificity	Porcine origin. High purity and activity	Porcine origin. Available lyophilized or frozen	Enhanced cleavage efficiency. Tolerant to inhibiting agents	Rapid results Automation-friendly	Rapid results. Enhanced cleavage efficiency Automation-friendly	Suppression of deamidation and disulfide scrambling

ProteaseMAX™ - MS Compatible Surfactant

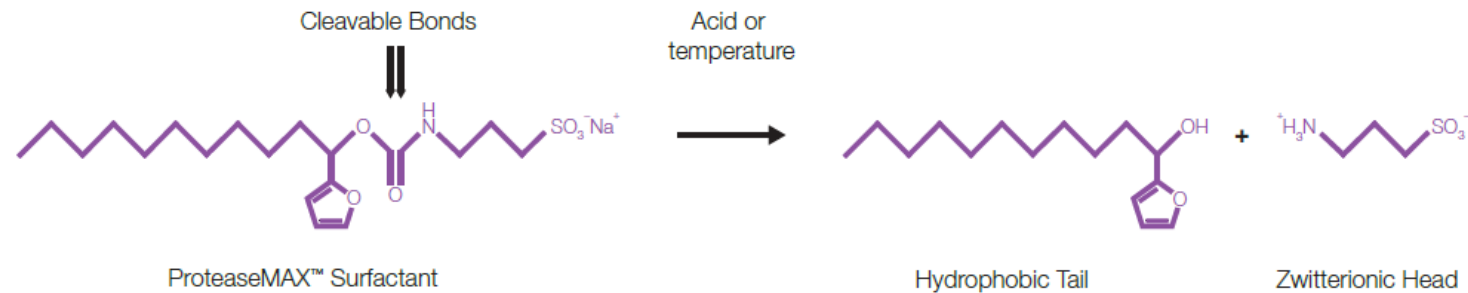


ProteaseMAX™ - MS Compatible Surfactant

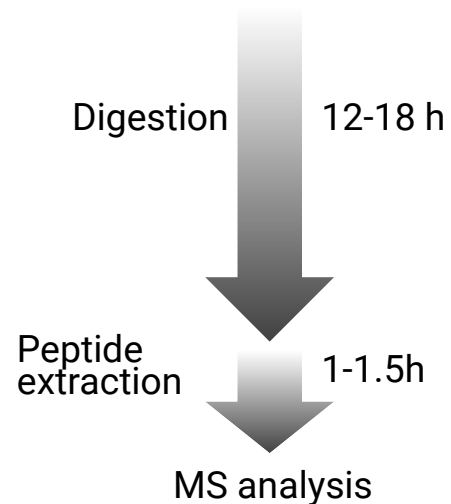
- Improves in-gel and in-solution protein digestion
- Denatures and solubilizes proteins
- Degrades over the course of the digestion reaction, yielding products that are compatible with mass spectrometry



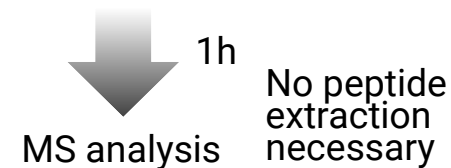
ProteaseMAX™ in-gel digestion



Conventional **In gel** digestion



ProteaseMAX™-assisted in-gel digestion

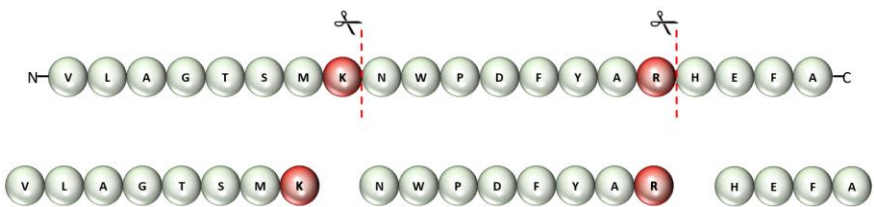


Alternative Proteases

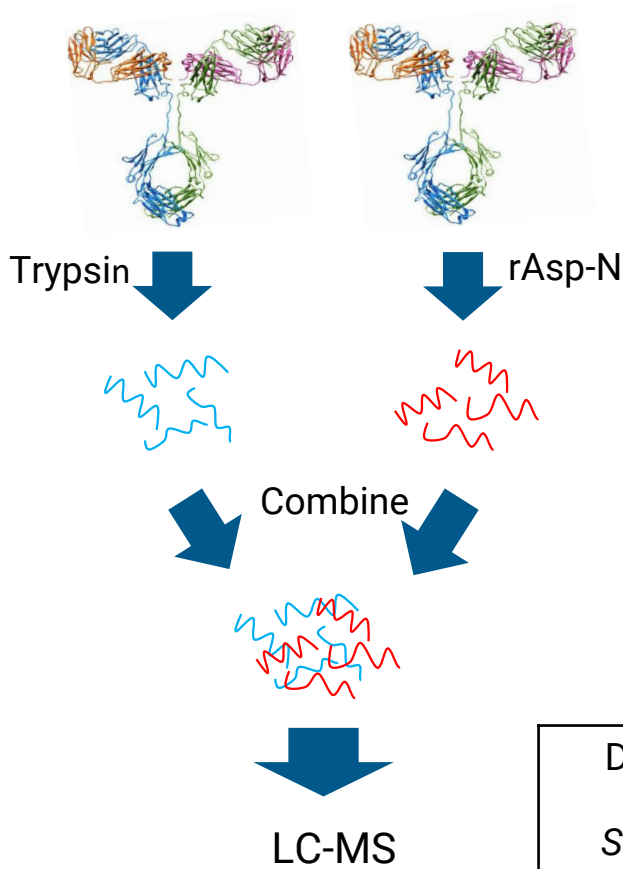
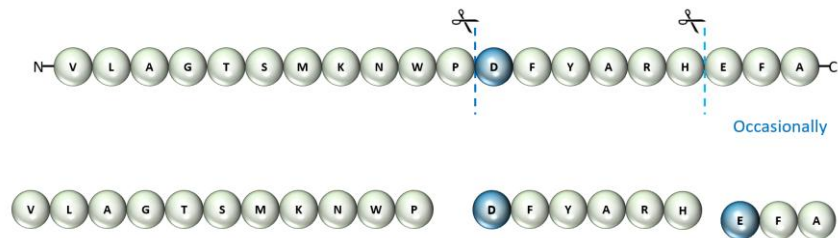


Why using alternative proteases?

Trypsin cleavage specificity



Asp-N cleavage specificity

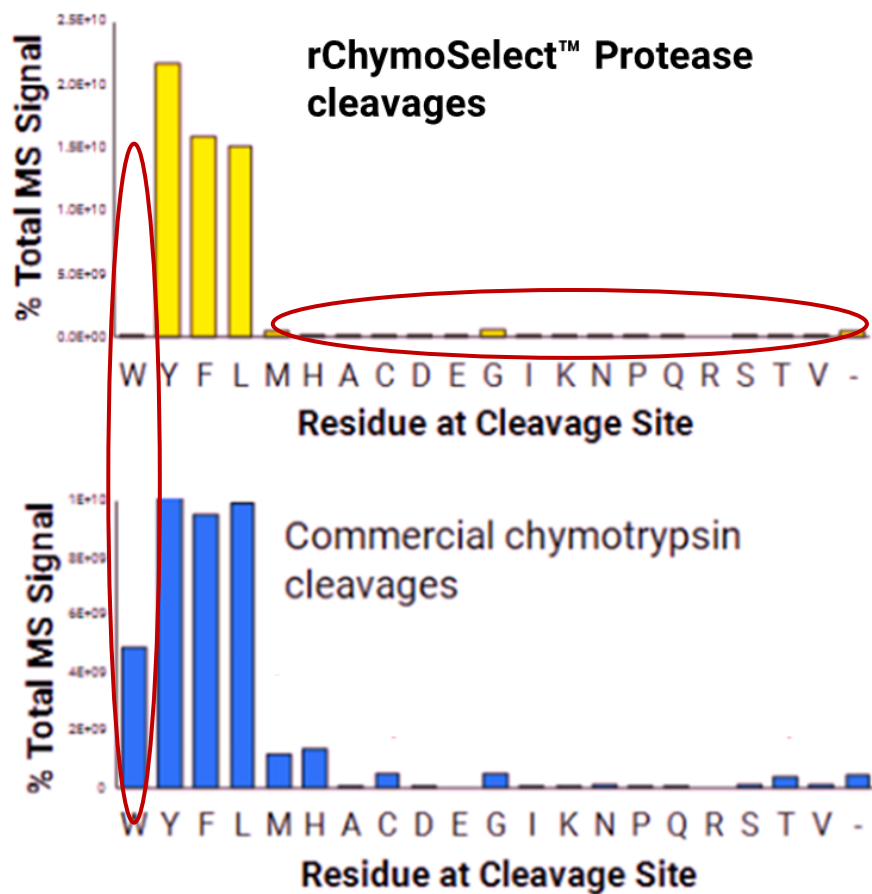


Digest	Sequence Coverage		
	rAsp-N <i>Asp-N</i>	Trypsin <i>Trypsin</i>	Combined <i>Asp-N & Trypsin</i>
Heavy Chain	94	88	100
Light Chain	89	80	100

Alternative Proteases

Proteases	Cleaves after	pH	Source
rChymoSelect™, Mass Spec Grade	Y, F, L		Recombinant <i>Pichia pastoris</i>
Arg-C Ultra, Mass Spec Grade	R	5.5 – 9.0	<i>Porphyromonas gingivalis</i>
rAsp-N, Mass Spec Grade	Before D	6.0 – 9.0	Recombinant <i>E. coli</i>
rLys-C, Mass Spec Grade	K	8.0 – 9.0	Recombinant <i>E. coli</i>
Lys-C, Mass Spec Grade	K	7.0 – 9.0	<i>Lysobacter sp.</i>
ProAlanase, Mass Spec Grade	P, A	1.0 – 5.5	<i>Aspergillus niger</i>
Arg-C, Sequencing Grade	R, K	7.6 – 7.9	<i>Clostridium histolyticum</i>
Asp-N, Sequencing Grade	Before D	4.0 – 9.0	<i>Pseudomonas fragi</i>
Chymotrypsin, Sequencing Grade	Y, F, W	7.0 – 9.0	Bovine Pancreas
Glu-C, Sequencing Grade	E	4.0 – 9.0	<i>Staphylococcus aureus</i> V8

rChymoSelect Protease, Mass Spec Grade



Does not cleave after **Trp**, enabling accurate analysis of **Trp** oxidation

Eliminates non-specific cleavages

Free from trypsin contamination

Resistant to autoproteolysis for cleaner peptide maps

Arg-C Ultra, Mass Spec Grade

Recombinant C-terminal Cysteine protease

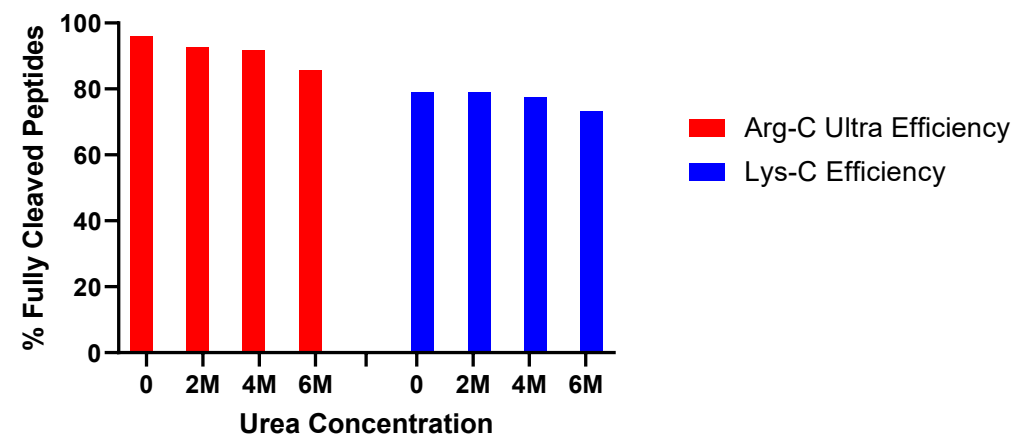
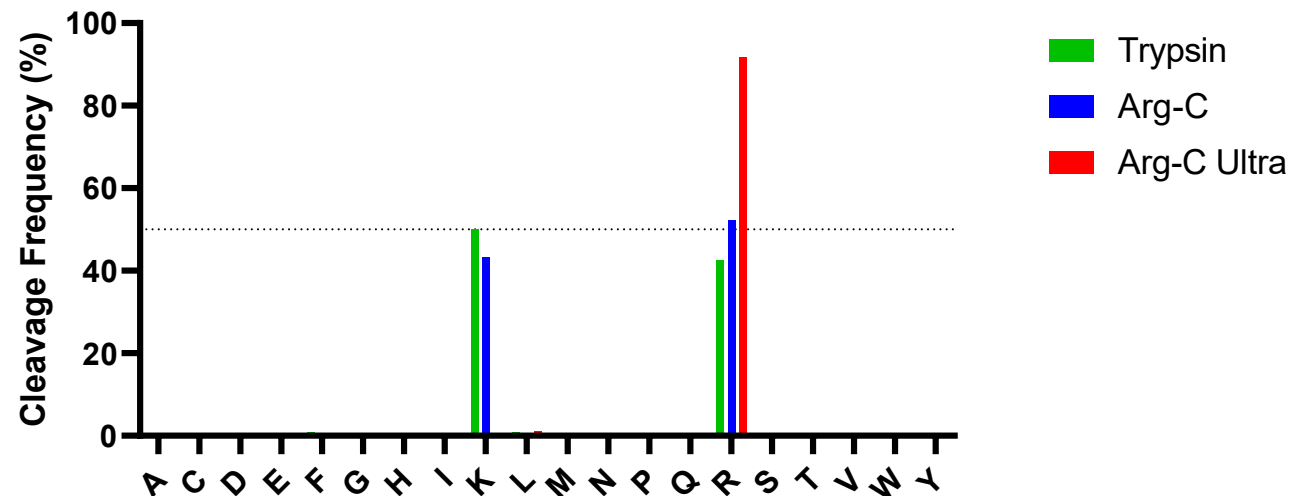
cleaves exclusively at the C-terminus of arginine residues

Highly Processive even in 6M urea

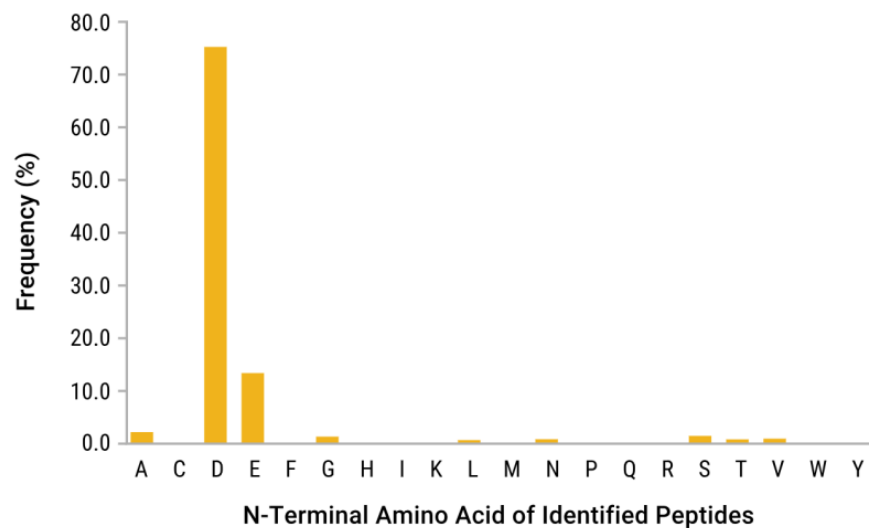


Protocols for Sequential or Simultaneous Arg-C Ultra/Lys-C digests in [Technical Manual TM754](#)

C-Terminal Cleavage Specificity

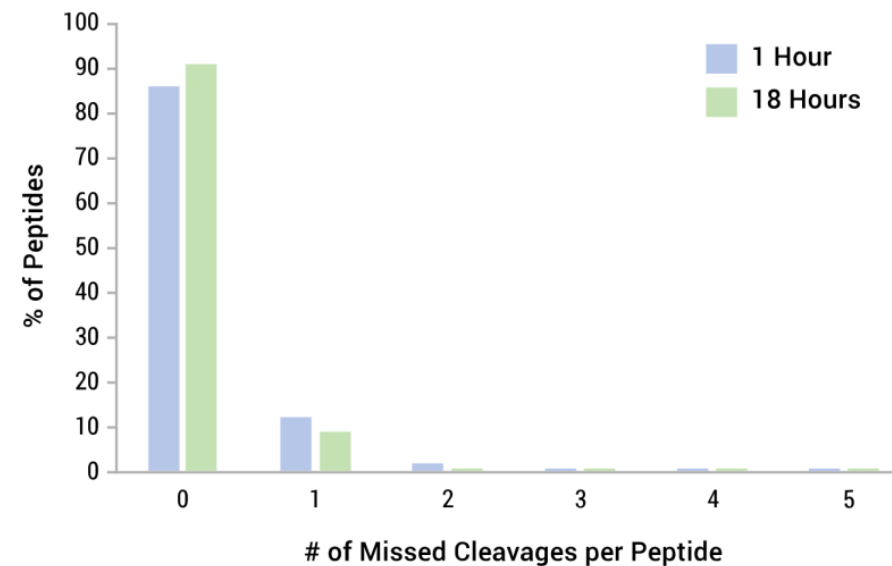


rAsp-N, Mass Spec Grade

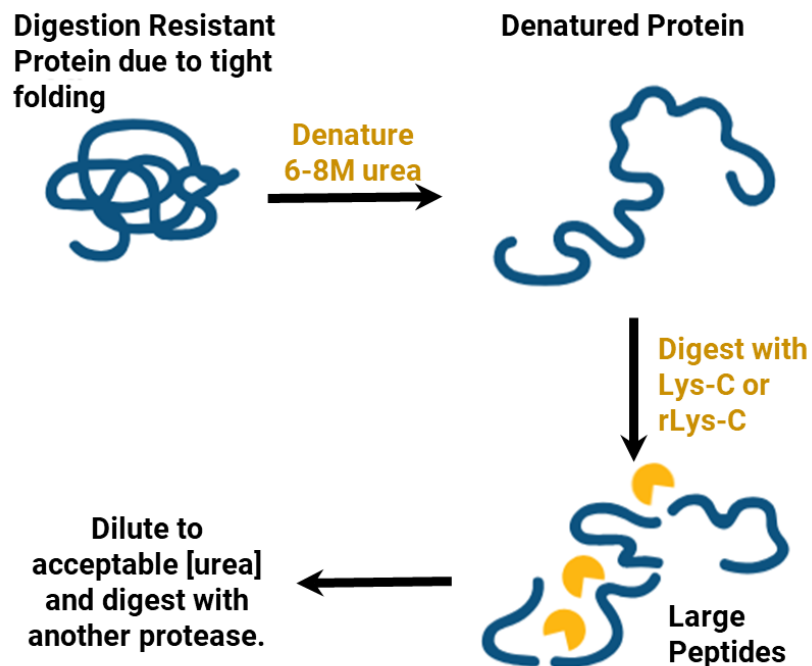


Efficient Protein Digestion in 1 Hour

Digestion N-Terminal to Aspartic Acid Residues

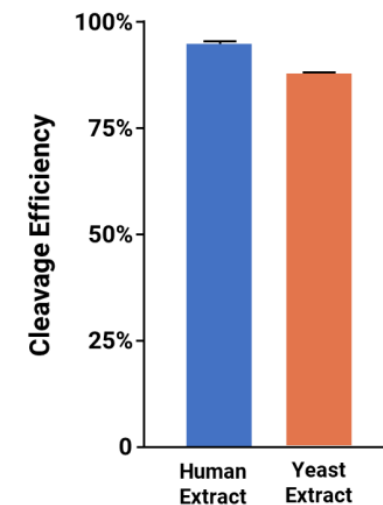
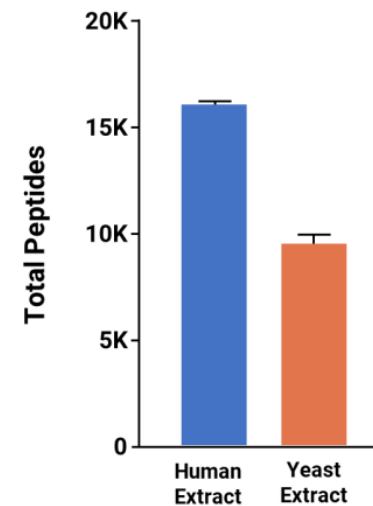
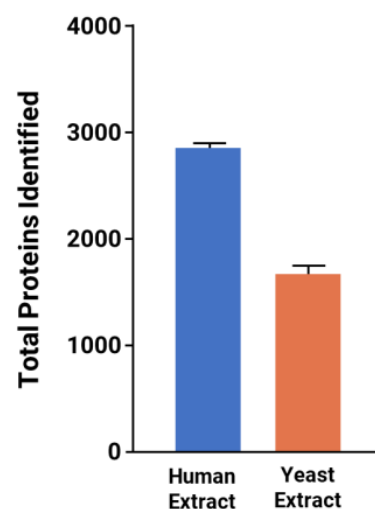


Lys-C, Mass Spec Grade



Make tightly folded protein analysis easier

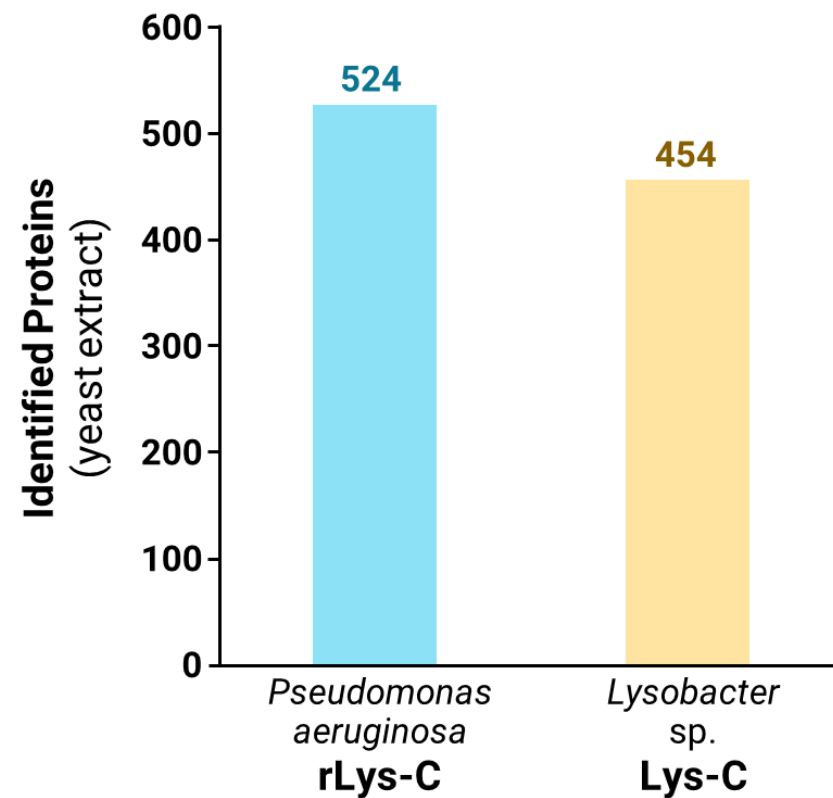
Digest Complex Protein Mixtures



rLys-C, Mass Spec Grade

Paired with Trypsin Gold in Trypsin/Lys-C Mix

Comparable Proteome Coverage to native Lys-C

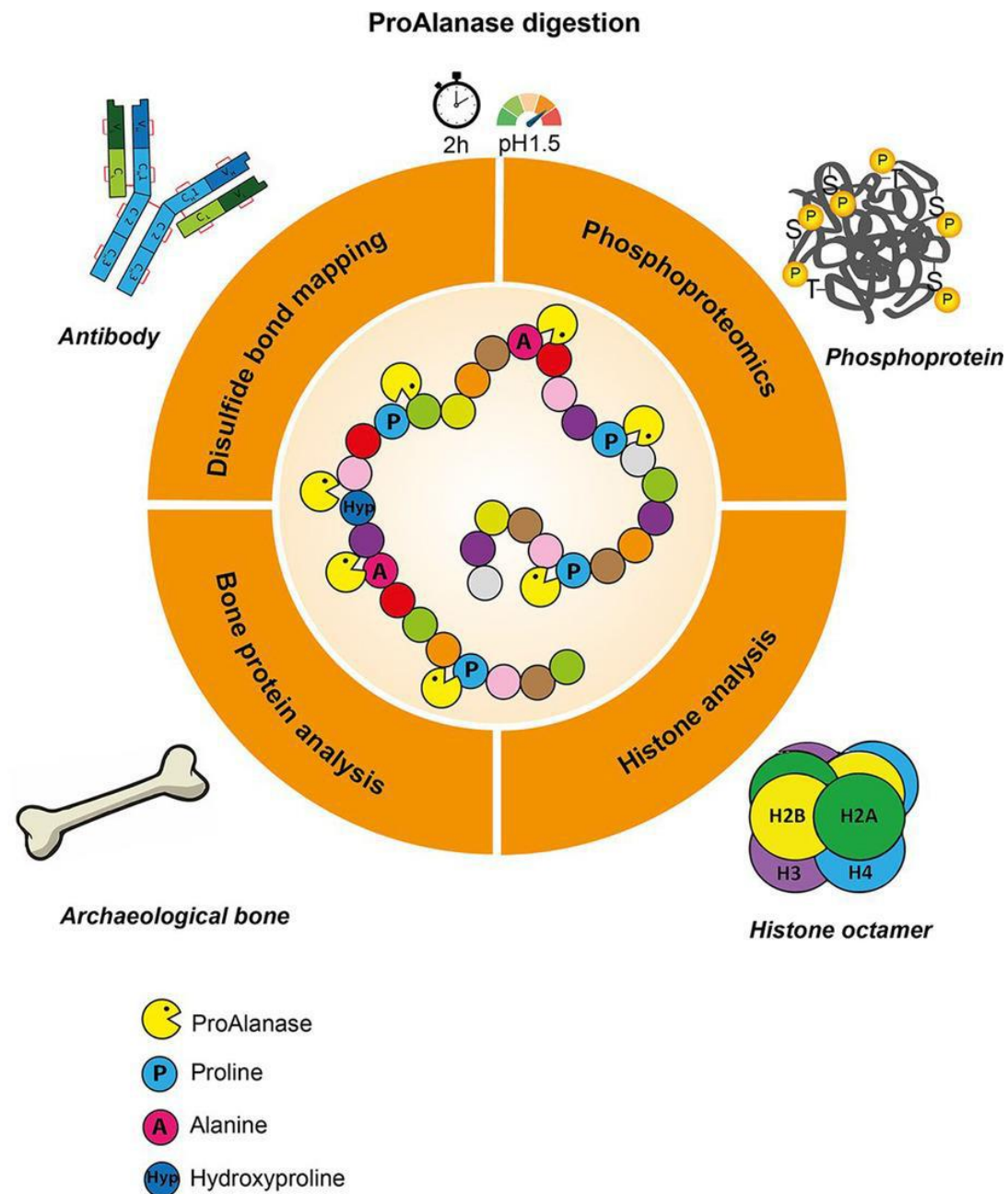
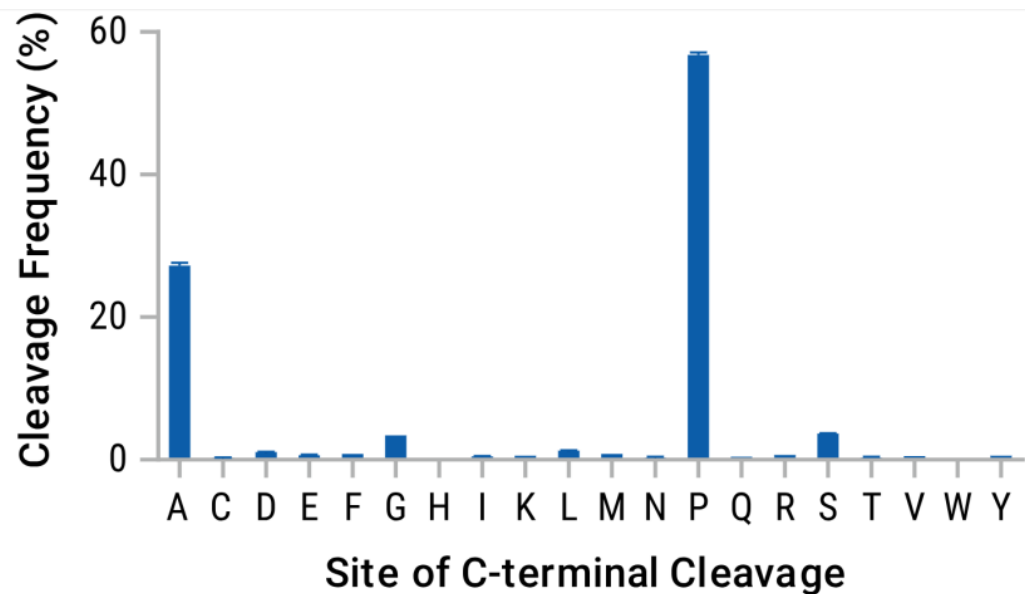


ProAlanase, Mass Spec Grade

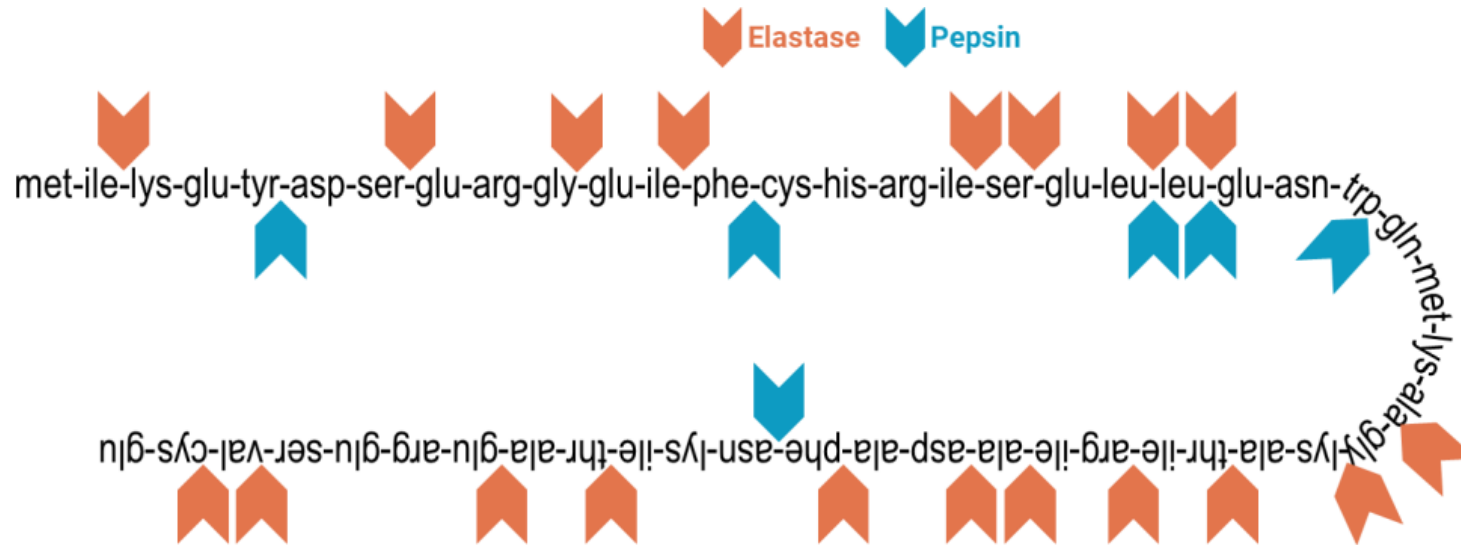
C-terminal cleavage specificity of ProAlanase

Optimal Performance with Short Digestion Times

Optimal Digestion at pH 1.5



Broad Spectrum Proteases



Elastase:

- purified from porcine pancreas
- cleaves at the C-terminus of alanine, valine, serine, glycine, leucine or isoleucine
- is active at pH 9.0

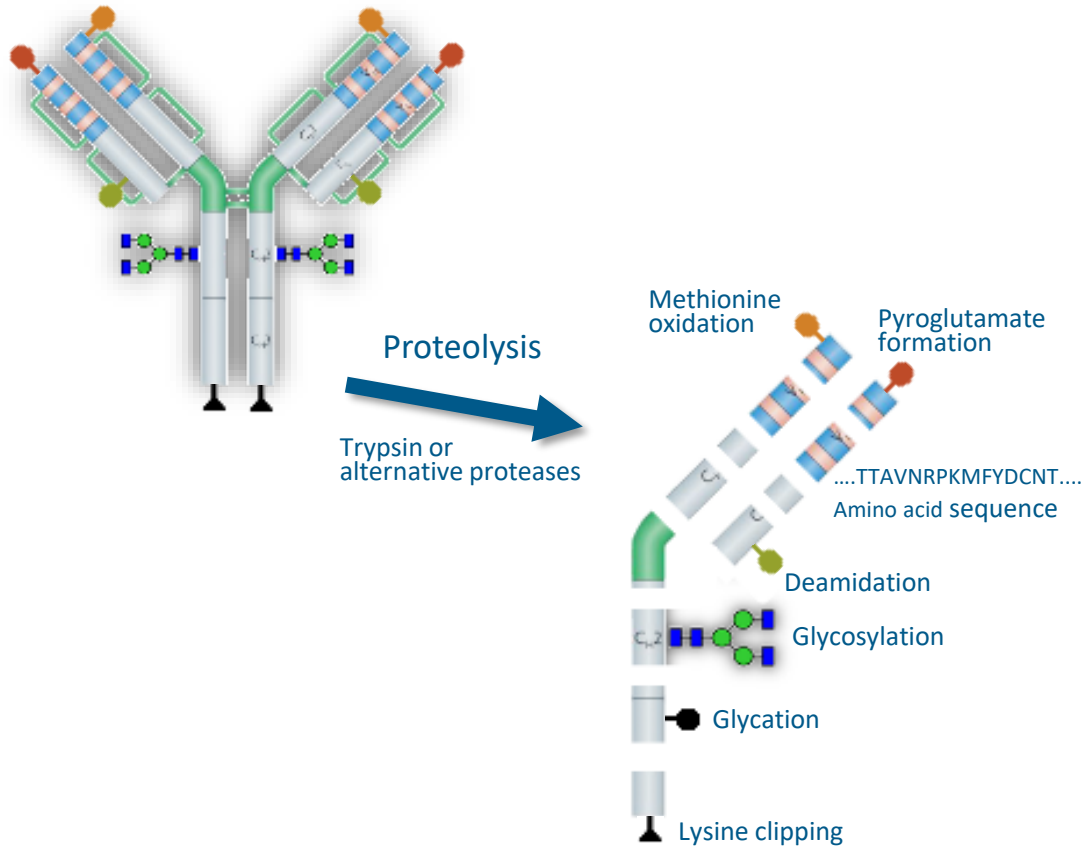
Pepsin:

- purified from porcine stomach
- cleaves at the C-terminus of phenylalanine, leucine, tyrosine and tryptophan
- is active at pH range 1.0 – 3.0

Mass Spec Proteases for Biotherapeutic Analysis



Peptide Mapping



Objectives of peptide mapping

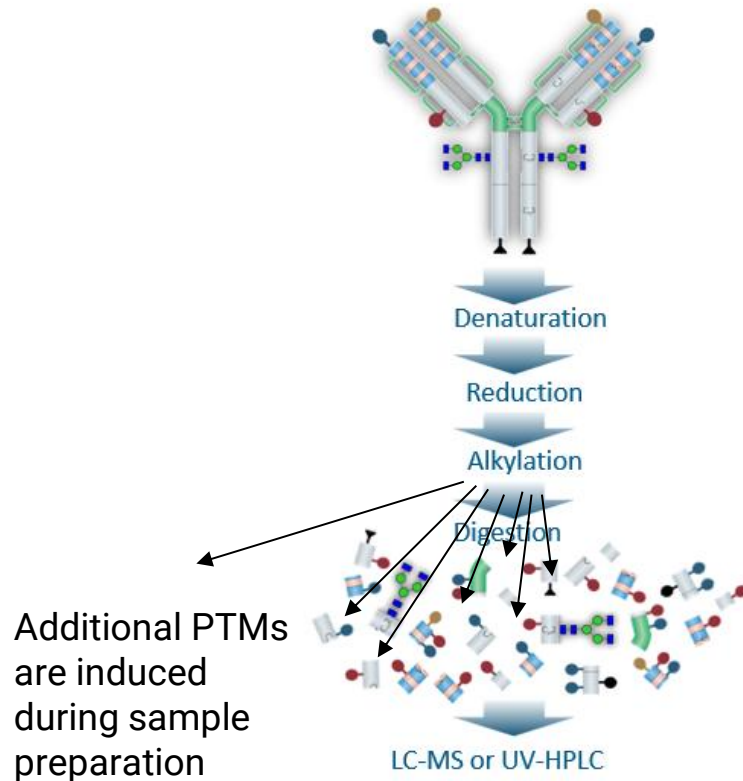
- Full amino acid sequence
 - Sequence variants
- Glycan analysis
- Disulfide bond mapping
- Lysine clipping
- Pyroglutamation, etc.
- **Non-enzymatic PTMs**

Non-enzymatic PTMS:

- Deamidation
- Disulfide Bond Scrambling
- Methionine Oxidation

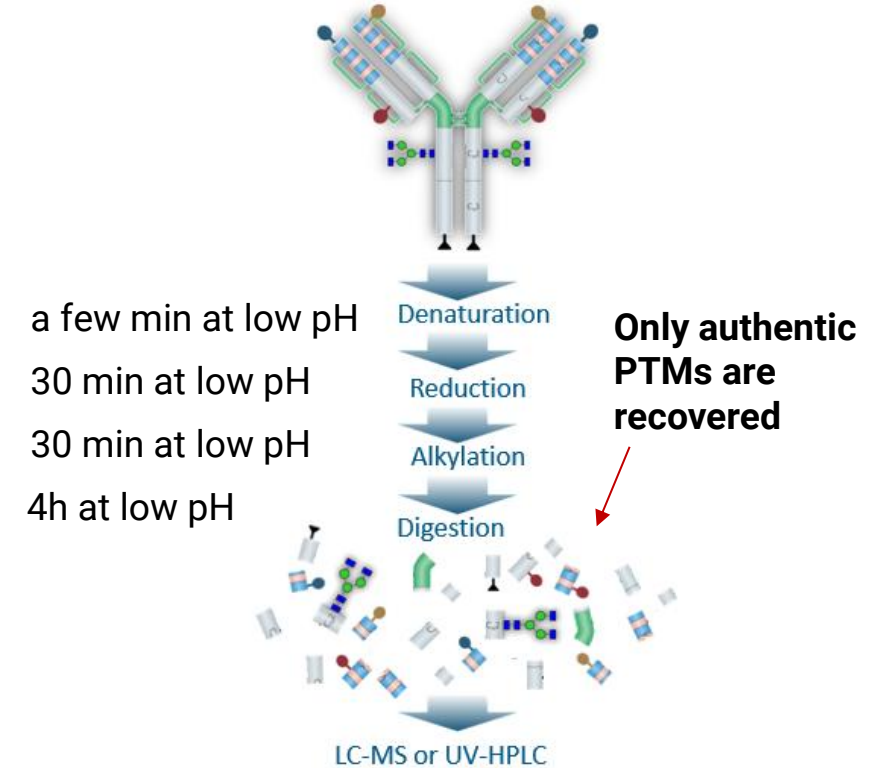
AccuMAP™ Low pH Protein Digestion kit

Conventional sample preparation



Traditional sample prep at alkaline pH introduce artificial PTM's

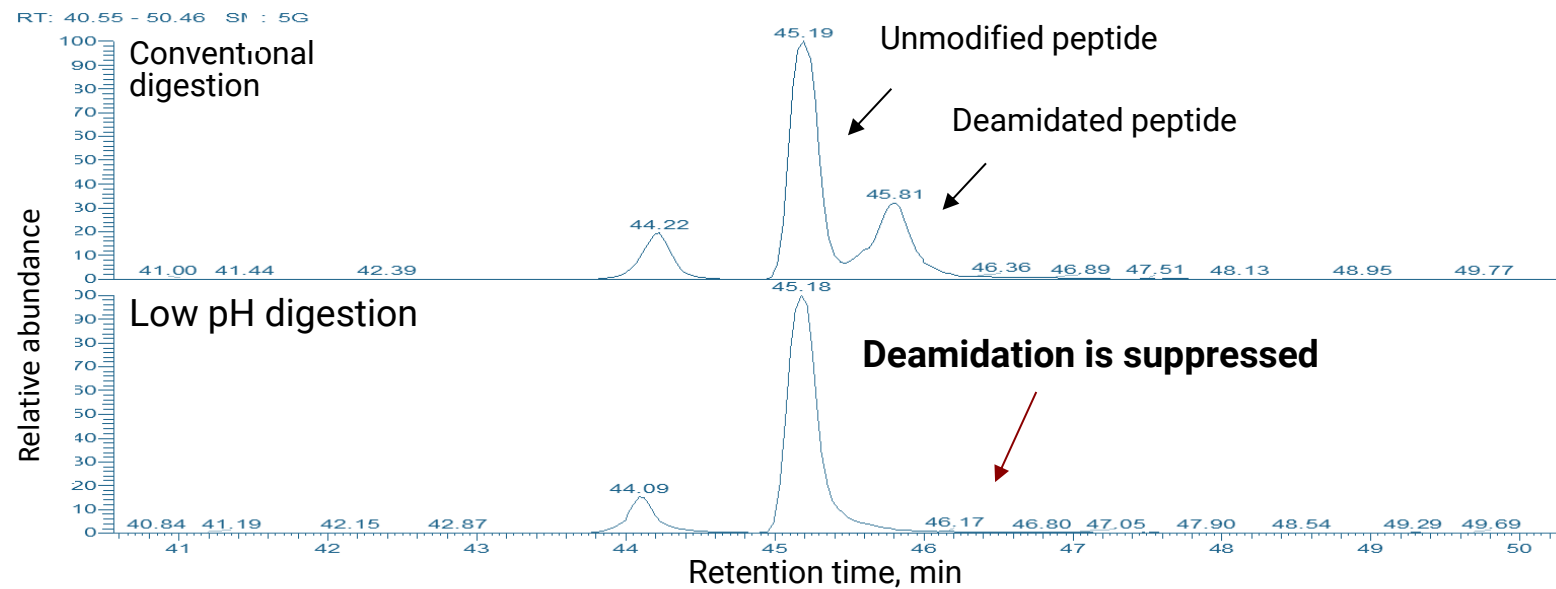
AccuMAP™-assisted sample preparation



Suppression of artificial PTMs

AccuMAP™ Low pH Protein Digestion kit

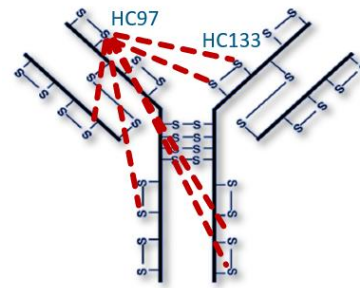
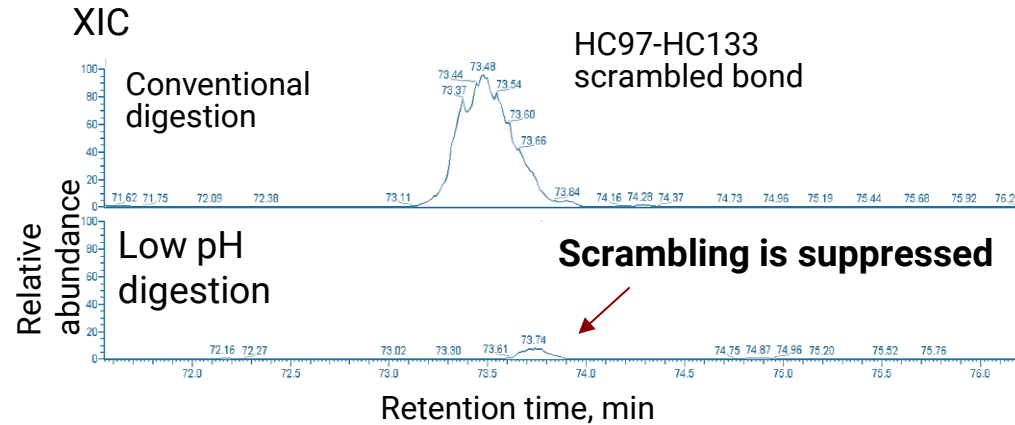
Suppression of sample prep-induced deamidation



Model IgG was digested according to conventional and low pH methods and analyzed with LC/MS

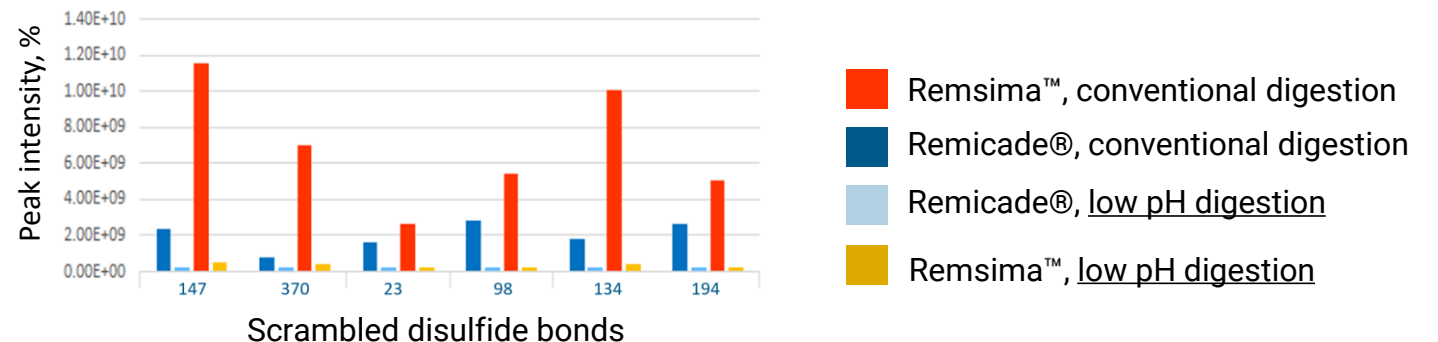
AccuMAP™ Low pH Protein Digestion kit

Suppression of disulfide bond scrambling



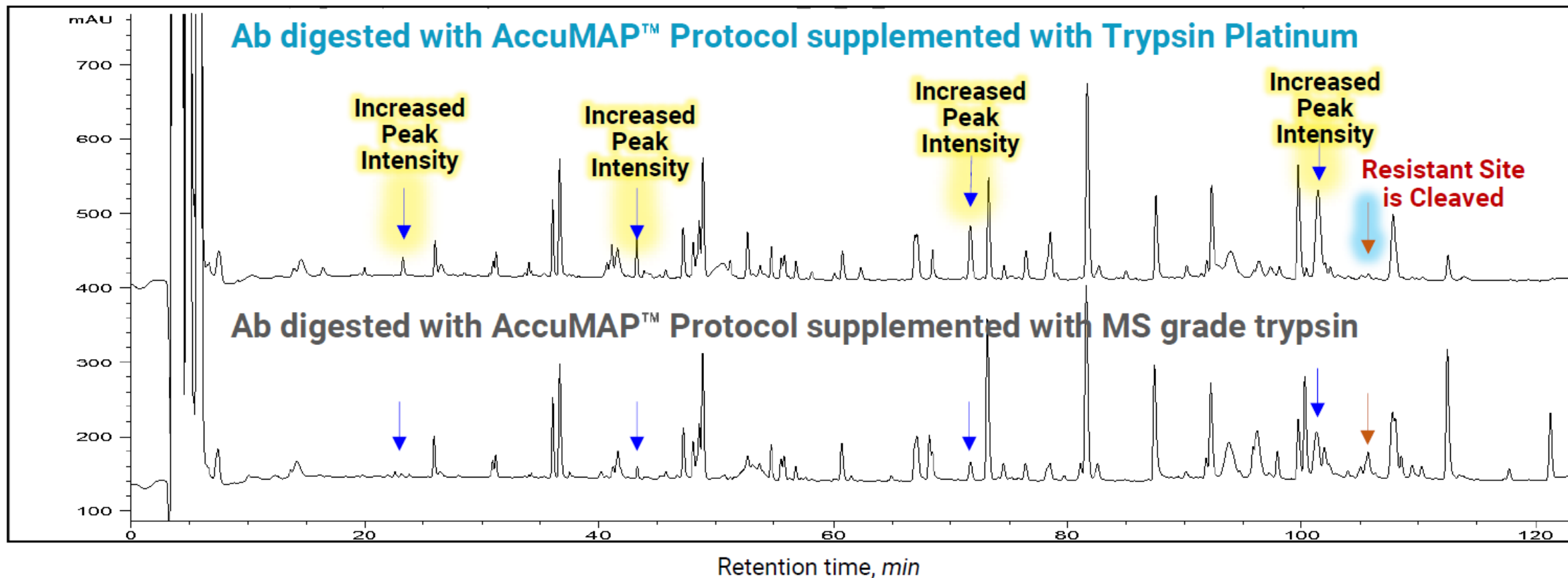
Study of disulfide bond scrambling in Remicade® and its BioSimilar Remsima™

LC-MS/MS analysis of the antibodies digested at conventional and low pH conditions



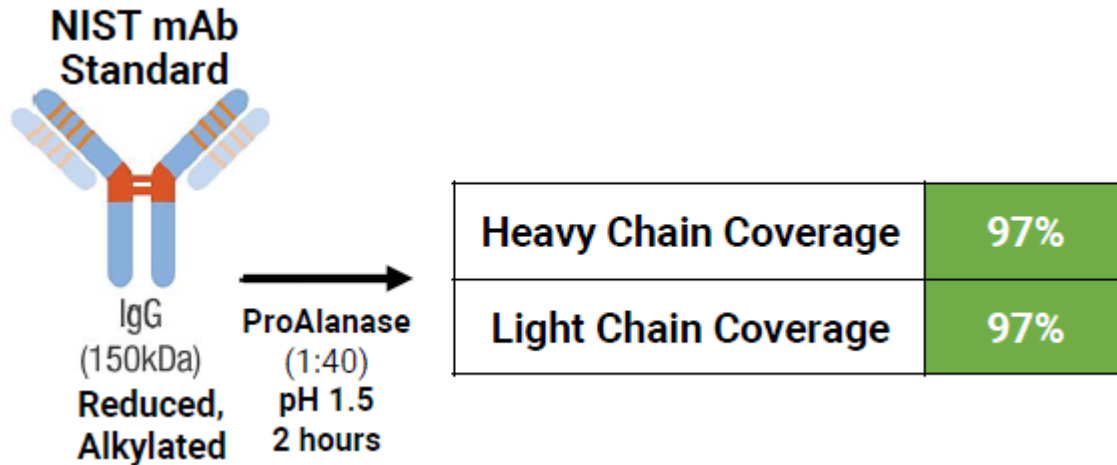
Study by U. Michigan, MS BioWorks, LLC, Protein Metrics Inc. and Promega Corporation

Trypsin Platinum for Biotherapeutic Analysis

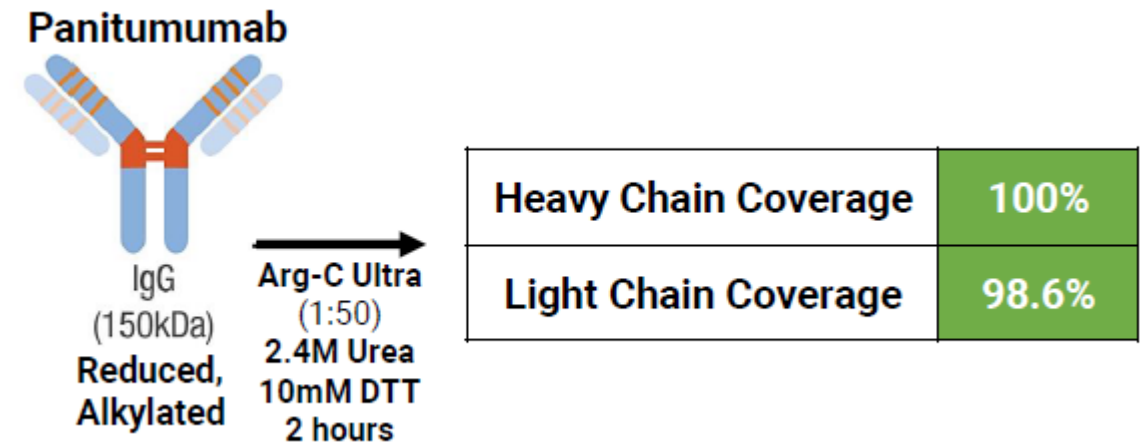


Additional Mass Spec Proteases for Biotherapeutic Analysis

ProAlanase, Mass Spec Grade

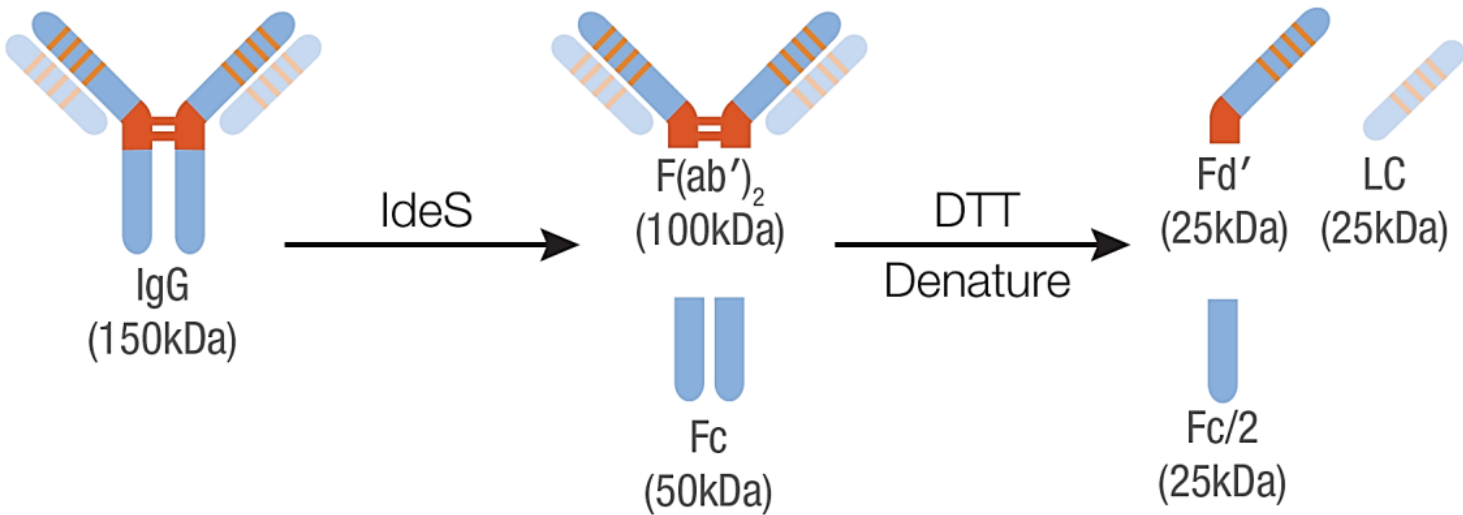


Arg-C Ultra, Mass Spec Grade



IgG Degrading Enzymes IdeS and IdeZ

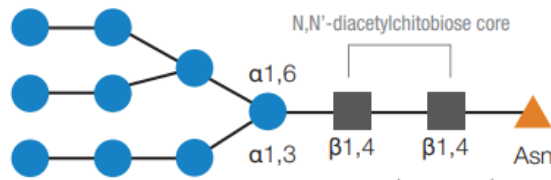
cleaves Immunoglobulin G (IgG) with high specificity at a single site below the hinge region
→ yielding F(ab')₂ and Fc fragments



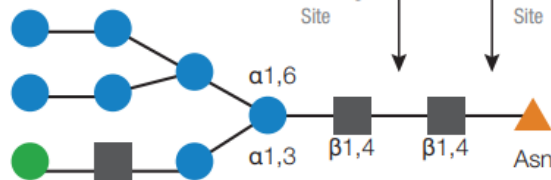
Subclass	Hinge/CH2 Sequence (cleavage site: ▼)	IdeS Activity	IdeZ Activity
Human IgG1	CPPCPAPELL G ▼ G PSVF	+++	+++
Human IgG2	CPPCPAPPV A ▼ G PSVF	+++	+++
Human IgG3	CPRCPAPELL G ▼ G PSVF	+++	+++
Human IgG4	AHHAQAPEFL G ▼ G PSVF	+++	+++
Mouse IgG1	PCICTVPEFSSVF	—	—
Mouse IgG2a	CPPCAAPNLL G ▼ G PSVF	+	+++
Mouse IgG2b	CHKCPAPNLEGGPSVF	—	—
Mouse IgG3	GSSCPAGNIL G ▼ G PSVF	+	+++

Glycosidases: PNGase F and Endoglycosidase H

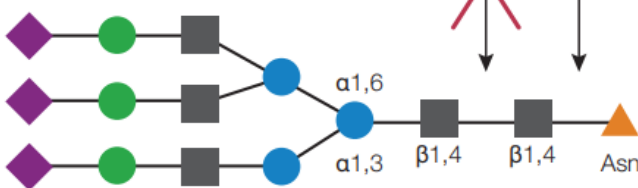
High Mannose Structure



Hybrid Structure



Complex Glycan Structure



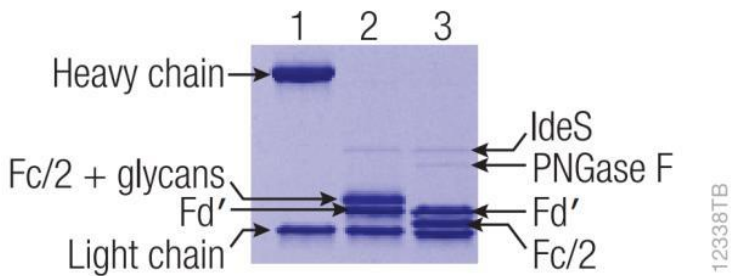
Legend:

- GlcNAc
- Galactose
- ▲ Fucose
- Mannose
- ◆ Sialic Acid

Endo H
Cleavage
Site

PNGase F
Cleavage
Site

Endo H is unable
to cleave N-linked
complex-type glycans



PNGase F plus IdeS in a single reaction



[View Google Scholar hits for "PNGase F" since 2024](#)



[View Google Scholar hits for "Endo H" since 2024](#)

Choosing the Right Protease

**STANDARD
WORKFLOW**

**Trypsin
Gold**

FAST

**Rapid
Trypsin**

**TOUGH
PROTEINS /
MAX
COVERAGE**

**Trypsin/
Lys-C Mix**

**BEYOND
TRYPSIN**

**Alternative
Proteases**

PHARMA

**AccuMAP +
IdeS/IdeZ
Trypsin
Platinum**

Match the tool to your application

Thank you

- For additional questions please contact:
kseniya.carr@promega.com
- For products & sales relevant information:
eastport@eastport.cz

