Title: Improved in-gel digestion results and work-flow through the use of a mass spectrometry compatible surfactant

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Abstract # ThP 101

Promega

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1. Introduction

Typical protocols for in-gel protein digestion require overnight incubation followed by additional 2 – 3 hours for peptide extraction. We present here a protocol and reagent to streamline this process and in many cases improve protein coverage.

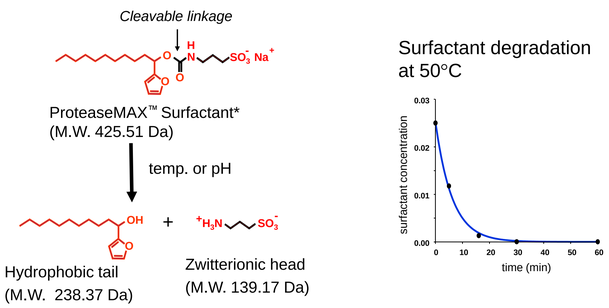
The reagent, ProteaseMAX™ surfactant, enhances peptide cleavage by providing a denaturing environment prior to protease addition. It is also designed to degrade during the proteolytic reaction, generating species that do not interfere with mass spectrometry.

For in-gel applications, complete digestions can be performed in 1 hour and the surfactant provides concurrent peptide extraction. This obviates the requirement for separate extraction processing, greatly simplifying the overall work-flow. Recovery is particularly enhanced for longer and more hydrophobic peptides (2,500-4,000Da), thereby improving coverage.

Enhanced solubilization by ProteaseMAX™ surfactant improves in-solution digestion of complex proteomic samples. Hydrophobic membrane proteins can be solubilized at room temperature in under 1 hr. Proteome coverage of a mouse membrane extract, analyzed by 2D LC-MS/MS, was increased 70% by incorporating the surfactant into the sample preparation protocol.

2. ProteaseMAX™ surfactant physical properties

ProteaseMAX™ is an anionic surfactant that is stable as a 1% stock solution at -20°C. However, under digestion conditions (e.g., 1h at 50°C, 0.025% surfactant) it degrades and loses its surfactant-like character. The degradation product does not interfere with LC or MS (Sections 3, 4 and 6).



3. Base peak chromatogram of In-gel digestion with ProteaseMAX™ surfactant

The 55kD region of an extract of mouse heart was excised and digested with ProteaseMAX™ surfactant and analyzed by LC-MS without further clean-up.

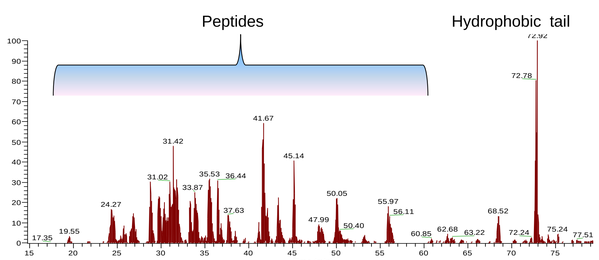


Figure: Base Peak Chromatogram shows the sharp elution of surfactant degradation product at end of the gradient is well resolved from peptides and does not interfere with MS analysis. Data collected on a Thermo LCQ.

4. Improved peptide recovery with surfactant assisted peptide extraction

HTR1A protein was expressed in Wheat germ lysate and purified with and without $^{13}C_6$-Arg labeling, normalized, run on a 1D SDS gel and digested for 1hr at 50°C either in the presence or absence of surfactant. Following digestion, reactions were mixed 1:1 and isotope ratios were used to demonstrate increased peptide recovery with the surfactant. Experiment was inverted to verify improvement with surfactant.

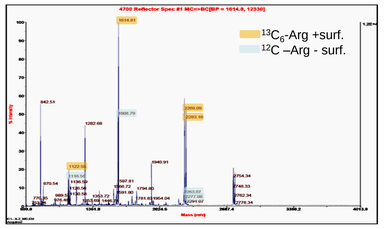


Figure: $^{13}C_6$-Arg labeled version of HTR1A with surfactant extraction (yellow shading) mixed 1:1 with ^{12}C-Arg HTR1A no surfactant extraction (blue shading).

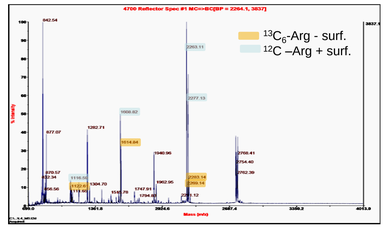


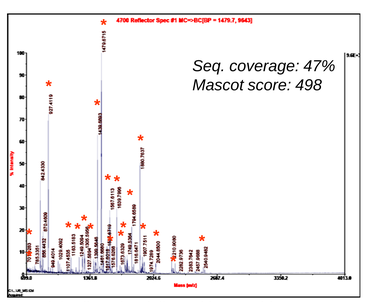
Figure: ^{12}C-Arg labeled version of HTR1A with surfactant extraction (blue shading) mixed 1:1 with $^{13}C_6$-Arg HTR1A no surfactant extraction (yellow shading).

5. Improved peptide recovery and protein coverage without extraction

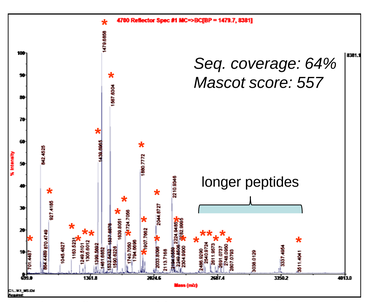
Differential recovery of peptides after in-gel digestion due to poor extraction and absorptive losses after extraction are a major limiting factor in obtaining the best sequence coverage possible.

- Surfactant assisted in-gel digestion improves the peptide release from the gel eliminating the need for post digestion extraction reducing process time and streamlining work-flow
- The presence of low level surfactant in the digestion reaction helps minimizes adsorptive losses of peptides after extraction
- Surfactant improves recovery of long peptides >2,500 Da to the levels suitable for MS/MS analysis
- *Improved peptide extraction, minimized peptide loss and improved recovery of long peptides have resulted in a more consistent, reliable in-gel digestion process*

6. Comparison of surfactant protocol with standard overnight protocol for a model protein (BSA).



Overnight digestion followed by peptide extraction



One hour digestion with ProteaseMAX™

Figure: BSA (50 ng) was in-gel digested with trypsin overnight or 1h in the presence of ProteaseMAX™. Digests were extracted with C-18, spotted and analyzed on an AB 4800 MALDI-TOF/TOF. Asterisks indicate identified peptides.

7. Comparison of conventional overnight and surfactant assisted protocols for in-gel digestion of various Human and Mouse proteins

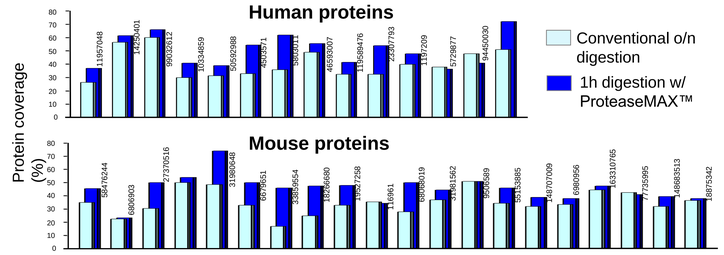


Figure: Human or mouse proteins from cytoplasmic and membrane extracts were resolved with SDS-PAGE, stained with Coomassie Blue, in-gel digested and analyzed with an AB 4800 MALDI-TOF/TOF. Proteins are identified by gi # at the right

- *Higher protein coverage was achieved with ProteaseMAX™ surfactant in most cases due in part to improved recovery of longer peptides.*

8. Other examples of the application of ProteaseMAX™ surfactant

In solution complex protein digestion:

Rod K Nibbe, et. al.
Discovery and scoring of protein interaction sub-networks discriminative of late stage human colon cancer
Mol. Cell. Proteomics, Apr 2009; 8: 827 - 845

Samuel K. Campos[a] and Michelle A. Ozbun[*]
Two Highly Conserved Cysteine Residues in HPV16 L2 Form an Intramolecular Disulfide Bond and Are Critical for Infectivity in Human Keratinocytes PLoS ONE. 2009; 4(2): e4463

Xiaotao Duan , et al.
A straightforward and highly efficient precipitation/on-pellet digestion procedure coupled to long gradient nano-LC separation and Oribtrap mass spectrometry for the label-free expression profiling of swine heart mitochondria proteome *J. Proteome Res.*, Articles ASAP, Publication Date (Web): March 16, 2009 (Article)
DOI: 10.1021/pr900001t

In-gel complex protein digestion:

Chris Adams, et al.
In-Gel Digestion of Mouse Membrane Protein Extract: 85% Increase in Peptide Recovery and Identification of Very Low Abundance Hydrophobic Proteins *57th ASMS Conference on Mass Spectrometry*, # **ThP 526**

9. Summary

Improved sample preparation for mass spectrometry with the new ProteaseMAX™ surfactant

- ❑ In-gel protein digestion and peptide extraction in a single one-hour step
- ❑ Recovery of longer and more hydrophobic peptides from polyacrylamide gels (2,500-4,000 Da), providing improved protein coverage
- ❑ Improved coverage of membrane proteome owing to enhanced protein solubilization and synergy with Urea
- ❑ Degradation over the course of a protein digestion allowing sample analysis without further clean-up or extraction

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