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Tips from our Protein Separation ZenMasters

Peptide Mapping

Chad Eichman, Ph.D.
BioPharm Global Marketing Manager

How long should my trypsin digest take?

This answer depends on the context of the analysis. In proteomics applications, long digests (e.g. 8 hours) can often yield more reproducible results, while longer digests exceeding 16 hours will almost invariably lead to drops in sequence/proteome coverage because of nonspecific activity of trypsin. It is also dependent on the enzyme to substrate ratio; a higher enzyme to substrate ratio might work well for faster digestions, but might lead to more nonspecific cleavages.[1]

Although this is sample specific, if turnaround time is an issue, faster digestion times might be appropriate, especially for "conforms to standard" applications. This is especially true for mAbs; a 3 hour digestion at 37 °C might be as effective as a digestion performed at 55 °C for 1 hour.[2] As mentioned previously, a higher enzyme to substrate ratio (e.g. 1:10) might be more appropriate here.

Alternatively, for protein characterization, if an orthogonal enzyme is not used for sequence coverage, relying on trypsin missed cleavages might be a counterintuitive approach. Many short tryptic peptides are yielded by lysines (or possibly arginines) that are in close proximity in primary sequence, which will be cleaved if trypsin is allowed to digest the protein to completion. By decreasing the digestion time and accounting for the missed cleavages, you can increase sequence coverage. Again, counterintuitive but somewhat helpful in practice. Either way, with tryptic digests, consistency is key, and keeping digestion times consistent can ensure reproducible data.

What pH should my trypsin digest be performed at?

The pH needs to be experimentally determined but there are a couple of things to consider. Trypsin activity is optimal around pH 8.0. Commonly, for proteomics, pH can be critical for coverage so ammonium bicarbonate can be used as the digestion buffer. However, asparagine deamidation (and subsequent succinimide and asp/iso-asp rearrangement) could be a concern at this slightly basic pH.[3] This is sequence dependent, with the "NG" motif being the most prone to deamidation. As such, it might be better to run at a slightly lower pH (e.g. pH 7.5 or pH 7.6) to prevent deamidation.

MS grade stuff is expensive....Do I really need high quality reagents when performing my tryptic digest?

Yes, quality matters with trypsin digests! Pony up the money for MS-grade everything. High quality MS-grade solvents, MS grade trypsin, even high-quality reagents like iodoacetamide can go a long way. Even using low-protein binding microcentrifuge tubes and labware, can also incrementally improve data and reproducibility.

All incremental differences - high quality reagents, high quality labware, and consistency in sample preparation, can lead to a more robust peptide mapping method.

Should I perform a sample clean up before my LC-MS peptide mapping application?

For proteomics, using a clean up step (e.g. ZipTips® or solid phase extraction) might work well to increase sensitivity by removing ion suppressing reagents involved with the initial digest. However, you may lose sample anyway, so it may be a trade off between sensitivity and recovery. Of course, the other consideration is maintenance of the MS, as any cleanup step generally is a good practice, but if the intent is increase in sensitivity, the enrichment step might require additional optimization that might not be worth the effort.

My deconvolution software said that I get 98.7% sequence coverage of my monoclonal antibody! That's great, right?

It is always important to understand the algorithm by which your software is calculating a "hit" or identified peptide. This is typically calculated with a score of identified theoretical fragments using MS/MS.

Again, depending on the algorithm, this percentage may or may not accurately identify the peptide and could be a false positive. In other words, the software may not be confirming the MS/MS, only looking at m/z of the deconvoluted peptide fragment. Therefore, it becomes prudent to manually check b and y ion spectra, ensuring the number of theoretical fragments have indeed been identified.

1. Li F, Schmerberg CM, Ji QC. Accelerated tryptic digestion of proteins in plasma for absolute quantitation using a protein internal standard by liquid chromatography/tandem mass spectrometry. Rapid communications in mass spectrometry : RCM. 2009;23(5):729-732. doi:10.1002/rcm.3926.

2. Dick LW, Mahon D, Qiu D, Cheng KC. Peptide mapping of therapeutic monoclonal antibodies: improvements for increased speed and fewer artifacts. J Chromatogr B Analyt Technol Biomed Life Sci. 2009;877(3):230-6.

3. Hao P, Ren Y, Alpert AJ, Sze SK. Detection, Evaluation and Minimization of Nonenzymatic Deamidation in Proteomic Sample Prepaation. Molecular & Cellular Proteomics : MCP. 2011;10(10):O111.009381.

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allcrom@allcrom.com.br - www.allcrom.com.br
São Paulo (11) 3464 8900