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Recommendations for Different Proteases

S-Trap's™ work with your choice of protease.

Protease	Protease:Protein (w/w)	Digestion Time	Digestion Temperature	Digestion Buffer	Digestion Buffer for Isobaric Labeling
Asp-N[1]	1:20	3 hours	37 °C	0.5 mM $ZnCl_2$ in 50 mM NH_4HCO_3	0.5 mM $ZnCl_2$ in 50 mM TEAB
Chymotrypsin[2]	1:30	16-18 hours	37 °C	50 mM TEAB, 1 mM $CaCl_2$	
Glu-C[3]	1:10	16 hours	37 °C	0.1 M Phosphate Buffer, pH 7.7*	
Lys-C[4]	~1:30	4 hours	37 °C	Tris-HCl Buffer, pH 8.5	50 mM TEAB
Trypsin	1:10	16–18 hours	37 °C	50 mM TEAB, pH 8.5	

*Sodium phosphate must be removed prior to MS-analysis, especially when performing phospho-enrichment

Standard ProtiFi conditions are robust and different samples and experimental variations, especially targeted assays, may benefit from optimization.

Keep in mind:

1. If maximal enzyme/substrate performance is desired, for example to obtain maximum liberation of a target MRM peptide, hold the sample type and amount constant, and test different enzyme:substrate ratios (e.g. 1:10, 1:50, and 1:100 at different times (e.g. 1- 3 hours).
2. A typical protease:protein substrate ratio is 1:20 (w/w). A range of 1:10-1:100 can be implemented.
3. A typical incubation period at 37 °C is 4- 16 hours (overnight). A temperature of 47 °C for a shorter duration (1-2 hours) can also be used, enzyme dependent.
4. Specifics for your process must be tested accordingly.
5. Proteases:
    a. Asp-N (Reference: Tarentino, A. L., et al. Molecular Cloning and Sequence Analysis of Flavastacin: An O-Glycosylated Prokaryotic Zinc Metalloendopeptidase. Archives of Biochemistry and Biophysics, 319 (1): 281-285 (1995). https://doi.org/10.1006/abbi.1995.1293)
        i. Cleaves to the amino side of aspartic acid residues
        ii. Asp-N is a metalloprotease and requires small amounts of zinc to enhance activity
        iii. Maximal activity at pH 7.0- 8.0
    b. Chymotrypsin (Reference: Kostka, V. and Carpenter, F. H. Inhibition of Chymotrypsin Activity in Crystalline Trypsin Preparations. JBC, 239 (6): 1799-1803 (1963). https://doi.org/10.1016/S0021-9258(18)91261-5)
        i. Cleaves to the carboxyl side of tyrosine, phenylalanine, tryptophan, and leucine residues
        ii. Treatment with N-alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK) eliminates residual trypsin activity
    c. Glu-C (Reference: Liu. S., et al. Mildly Acidic Conditions Eliminate Deamidation Artifact during Proteolysis: Digestion with Endoprotease Glu-C at pH 4.5. Amino Acids, 48 (4): 1059-1067 (2016). https://doi.org/10.1007/s00726-015-2166-z)
        i. Cleaves the carboxyl side of glutamic acid
        ii. Activity and cleavage specificity is affected by buffer conditions