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
Lys-C[1]	~1:30	4 hours	37 °C	Tris-HCl Buffer, pH 8.5	50 mM TEAB
Asp-N[2]	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
Glu-C[3]	1:10	16 hours	37 °C	0.1 M Phosphate Buffer, pH 7.7*	
Chymotrypsin[4]	1:30	16-18 hours	37 °C	50 mM TEAB, 1 mM $CaCl_2$	
PNGase F (with trypsin)[5]	1:20	16–18 hours	37 °C	100 mM TEAB, 1 mM $CaCl_2$	

*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. Lys-C (Reference: Glatter, T., et al. Large-Scale Quantitative Assessment of Different In-Solution Protein Digestion Protocols Reveals Superior Cleavage Efficiency of Tandem Lys-C/Trypsin Proteolysis over Trypsin Digestion. J. Proteome Res. 11 (11): 5145–5156 (2012). https://doi.org/10.1021/pr300273g)
        i. Cleaves to the carboxyl side of lysine residues
        ii. Resistant to chemical denaturation
        iii. Active at pH 8.0- 9.5
        iv. Compatible with digestions up to 8 M urea
    b. 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
    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

d. 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

e. PNGase F (Reference: DeRosa, C.M., et al. Simultaneous N-Deglycosylation and Digestion of Complex Samples on S-Traps Enables Efficient Glycosite Hypothesis Generation. *ACS Omega* 8 (4): 4410-4418 (2023). https://doi.org/10.1021/acsomega.2c08071)
   i. Cleaves to the internal glycoside bond between the asparagine residue and innermost monosaccharide of the *N*-glycan, *N*-acetylglucosamine
   ii. Can hydrolyze high-mannose, hybrid, bi-, tri-, and tetra-antennary oligosaccharides
   iii. Deamidation modification detected by a mass shift + 0.984 at the deamidation site
   iv. Active at pH 7.0- 9.0

[1] Reference: Zougman, A., et al. Suspension trapping (STrap) sample preparation method for bottom-up proteomics analysis. Proteomics, 14 (9): 1006-1010 (2014). https://doi.org/10.1002/pmic.201300553

[2] Reference: Chavan, S., et al. Detecting red blood cell protein antigens by tandem mass spectrometry. Transfusion (2025). https://doi.org/10.1111/trf.18252

[3] Reference: Anmangandla, A., et al. The Acyl-CoA Specificity of Human Lysine Acetyltransferase KAT2A. Biochemistry, 61 (17): 1874-1882 (2022). https://doi.org/10.1021/acs.biochem.2c00308

[4] Reference: Stiving, A.Q., et al. Functionality and translation fidelity characterization of mRNA vaccines using platform based mass spectrometry detection. *npj Vaccines* 10, 38 (2025). https://doi.org/10.1038/s41541-025-01082-4

[5] Reference: DeRosa, C.M., et al. Simultaneous N-Deglycosylation and Digestion of Complex Samples on S-Traps Enables Efficient Glycosite Hypothesis Generation. *ACS Omega* 8 (4): 4410-4418 (2023). https://doi.org/10.1021/acsomega.2c08071