



### Storage/Stability

Solutions in 1 mM HCl (pH 3) are stable for approximately 1 year when aliquoted and stored at -20 °C. The presence of Ca<sup>2+</sup> (20 mM) will also retard trypsin's ability to selfdigest itself (autolysis) and will maintain the stability of the trypsin in solution.<sup>1,10</sup> Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.<sup>11</sup> Trypsin is reversibly denatured at high pH (above 11), by precipitation with TCA, or by high concentrations of urea (greater than 6.5 M).<sup>3</sup> In order to abolish all trypsin activity, heating at 100 °C in 1% (w/v) SDS for 5 minutes is required.<sup>12</sup>

### Procedure

For trypsin digestion of peptides, use a ratio (w:w) of about 1:100 to 1:20 for trypsin:peptide. Trypsin preparations usually contain some contaminating chymotrypsin and this should be inhibited with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK).<sup>9</sup>

### References

1. Walsh, K. A., Trypsinogens and trypsins of various species. *Meth. Enzymol.*, **19**, 41-63 (1970)
2. Buck, F. F., et al., On the mechanism of enzyme action. LXXII. Studies on trypsins from beef, sheep, and pig pancreas. *Arch. Biochem. Biophys.*, **97**, 417-424 (1962).
3. Keil, B., in *The Enzymes*, 3rd ed., Vol. III, Boyer, P. D., Academic Press (New York, NY: 1971), pp. 250-275.
4. Burdon, R. H., et al., in *Laboratory Techniques in Biochemistry and Molecular Biology*, Vol. 9, 2nd ed., Allen, G., ed., Elsevier/North (New York, NY: 1989), pp. 73-104.
5. *Enzyme Handbook*, Vol. II, Barman, T. E., Springer-Verlag (New York, NY: 1969), pp. 618-619.
6. USP, Vol. 23, p. 1611.
7. Wang, S. S., and Carpenter, F. H., Kinetics of the tryptic hydrolysis of the oxidized B chain of bovine insulin. *Biochemistry*, **6(1)**, 215-224 (1967).
8. *Proteolytic Enzymes, A Practical Approach*, Beynon, R. J., ed., IRL Press (New York, NY: 1989), p. 240.
9. Carpenter, F. H., Treatment of trypsin with TPCK. *Methods Enzymol.*, **11**, 237 (1967).
10. Sipos, T., and Merkel, J. R., An effect of calcium ions on the activity, heat stability, and structure of trypsin. *Biochemistry*, **9(14)**, 2766-2775 (1970).
11. *Methods of Molecular Biology*, Vol. 3, Smith, B. J., Humana Press, (New Jersey, 1988), pp. 57-69.
12. Porter, W. H., and Preston, J. L., Retention of trypsin and chymotrypsin proteolytic activity in sodium dodecyl sulfate solutions. *Anal. Biochem.*, **66**, 69-77 (1975).

TMG/RXR 5/06

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.