

## Product Information

### Trypsin TPCK treated from bovine pancreas aseptically filled

Catalog Number **T8802**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

CAS RN 9002-07-7  
EC 3.4.21.4  
Molecular mass:<sup>1,2</sup> 24 kDa  
Extinction Coefficient:<sup>3,4</sup>  $E^{1\%} = 12.9\text{--}15.4$  (280 nm)  
 $pI$ :<sup>2,5</sup> 10.1–10.5  
Synonyms: Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol

#### Product Description

Trypsin consists of a single chain polypeptide of 223 amino acid residues. Trypsin is produced by the removal of the N-terminal hexapeptide from trypsinogen, which is cleaved at the Lys<sup>6</sup>–Ile<sup>7</sup> peptide bond. The amino acid sequence of trypsin is crosslinked by 6 disulfide bridges. This native form of trypsin is referred to as  $\beta$ -trypsin. Autolysis of  $\beta$ -trypsin (which is cleaved at Lys<sup>131</sup>–Ser<sup>132</sup> results in  $\alpha$ -trypsin, which is held together by disulfide bridges). Trypsin is a member of the serine protease family. The active site amino acid residues of trypsin include His<sup>46</sup> and Ser<sup>183, 204</sup>.

Trypsin will cleave peptides on the C-terminal side of lysine and arginine amino acid residues. The rate of hydrolysis is slower if an acidic residue is on either side of the cleavage site and no cleavage occurs if a proline residue is on the carboxyl side of the cleavage site. The pH optimum of trypsin is 7–9.<sup>6</sup> Trypsin will also hydrolyze ester and amide linkages of synthetic derivatives of amino acids such as: benzoyl L-arginine ethyl ester (BAEE), *p*-toluenesulfonyl-L-arginine methyl ester (TAME), tosyl-L-arginine methyl ester, N $\alpha$ -benzoyl-L-arginine *p*-nitroanilide (BAPNA), L-lysyl *p*-nitroanilide, and benzoyl-L-arginamide.<sup>2,7,8</sup> Reported  $K_M$  values are BAEE (0.05 mM), TAME (0.05 mM), and BAPNA (0.94 mM).

Assuming the pH and temperature are the same and using a molar extinction coefficient of 808 at 254 nm for BAEE, the following conversions are valid:

1 BAEE  $\mu\text{M}$  Unit = 200 BAEE  $A_{253}$  Units  
1 TAME  $\mu\text{M}$  Unit = 0.27 BAEE  $\mu\text{M}$  Units  
1 BAEE  $\mu\text{M}$  Unit = 3.64 TAME Units  
1 TAME  $\mu\text{M}$  Unit = 55 BAEE  $A_{253}$  Units  
1 BAEE  $A_{253}$  Unit = 0.018 TAME  $\mu\text{M}$  Unit  
1 TAME  $\mu\text{M}$  Unit = 180 TAME  $A_{247}$  Units  
1 TAME  $A_{247}$  Unit = 0.33 BAEE Units  
1 USP Unit =  $\Delta A_{253}$  of 0.003 per minute  
1 NF Unit = 3.3  $A_{253}$  BAEE Units.<sup>9</sup>

The oxidized B chain of insulin is often used as a substrate to determine the suitability of trypsin for use in protein sequencing. The presence of two peptide bonds (Arg<sup>22</sup>–Gly<sup>23</sup> and Lys<sup>29</sup>–Ala<sup>30</sup>) make it an ideal peptide for use in this kind of application.<sup>10</sup>

Serine protease inhibitors that will inhibit trypsin include DFP (diisopropyl fluorophosphate), TLCK (N $\alpha$ -*p*-tosyl-L-lysine chloromethyl ketone), PMSF (phenylmethanesulfonyl fluoride), APMSF (4-amidinophenylmethane-sulfonyl fluoride), AEBSEF (4-(2-aminoethyl)benzenesulfonyl fluoride), aprotinin, leupeptin,  $\alpha_2$ -macroglobulin,  $\alpha_1$ -antitrypsin, *p*-aminobenzamidine, benzamidine (reversible), soybean trypsin inhibitor, lima bean inhibitor, bovine pancreas trypsin inhibitor, chicken egg white inhibitor, and turkey egg white inhibitor.<sup>2,11</sup>

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

This enzyme is soluble in 1 mM HCl (1 mg/ml), yielding a clear solution.

### Storage/Stability

Solutions in 1 mM HCl (pH 3) remain active for ~1 year when aliquoted and stored at -20 °C. The presence of calcium (20 mM) will also retard the autolysis of trypsin and maintain the stability of trypsin in solution.<sup>2,6</sup>

Trypsin retains most of its activity in 2.0 M urea, 2.0 M guanidine HCl, or 0.1% (w/v) SDS.<sup>13</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>14</sup>

### Procedure

For trypsin digestion of peptides, use a ratio (w/w) of 1:100 to 1:20 for trypsin:peptide. Trypsin preparations usually contain some contaminating chymotrypsin and should be inhibited with N-tosyl-L-phenylalanyl chloromethyl ketone (TPCK).<sup>12</sup> This product has been treated with TPCK to inhibit chymotrypsin activity.

### References

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SG, TMG, RXR, MAM 03/15-1