

## Product Information

### Trypsin from bovine pancreas

cell culture tested

Catalog Number **T9935**

Storage Temperature -20 °C

CAS RN : 9002-07-7

Enzyme Commission Number (EC): 3.4.21.4

Synonyms: Tryptase, Tryptar, Cocoonase, Parenzyme, Parenzymol

#### Product Description

Molecular Weight: 24 kDa<sup>1,2</sup>

Extinction Coefficient:  $E^{1\%} = 12.9 - 15.4$  (280 nm)<sup>3,4</sup>

pI: 10.1 - 10.5<sup>2,5</sup>

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 cross-linked 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>183,2,3,4</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  $\mu$ M Unit = 200 BAEE Units  
1 TAME  $\mu$ M Unit = 0.27 BAEE  $\mu$ M Units  
1 BAEE  $\mu$ M Unit = 3.64 TAME Units  
1 TAME  $\mu$ M Unit = 55 BAEE A<sub>253</sub> Units  
1 BAEE A<sub>253</sub> Unit = 0.018 TAME  $\mu$ M Unit  
1 TAME  $\mu$ M Unit = 180 TAME A<sub>247</sub> Units  
1 TAME A<sub>247</sub> Unit = 0.33 BAEE Units  
A USP Unit =  $\Delta A_{253}$  of 0.003 per minute  
1 NF Unit = 3.3 A<sub>253</sub> 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-amidinophenylmethanesulfonyl 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 Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Preparation Instructions

This enzyme is soluble in 1 mM HCl (1 mg/ml).

### Storage/Stability

Solutions in 1 mM HCl (pH 3) are stable for approximately 1 year when aliquoted and stored at  $-20\text{ }^{\circ}\text{C}$ . The presence of  $\text{Ca}^{2+}$  (20 mM) will also retard trypsin's ability to self digest itself (autolysis) and will maintain the stability of the 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  $100\text{ }^{\circ}\text{C}$  in 1% (w/v) SDS for 5 minutes is required.<sup>14</sup>

### Procedure

Trypsin may be used to remove adherent cells from a culture surface. Cells are most commonly removed from the culture substrate by treatment with trypsin, or trypsin•EDTA. Trypsin 1X solutions can range from 0.025% to 0.5%. The reasons for the range of concentrations are as follows: (1) Differences in trypsin activity or potency; (2) Different incubation times; (3) Different cell lines.

Incubating cells with too high a trypsin concentration for too long a time period will damage cell membranes and kill the cells. If unsure about the concentration of trypsin to use, use a low concentration. There can be lot-to-lot variation in dissociation times which is to be expected since the enzymatic activity of each lot will differ. If trypsin is being solubilized or diluted from a concentrated solution, it is important to use a buffered salt solution that contains no  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , such as Hank's Balanced Salt Solution, Modified (Product No. H 9394). Adjust the pH of trypsin solution to 7.4-7.6.

- 1) Remove medium from culture vessel by aspiration and wash the monolayer with  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  - free salt solution to remove all traces of serum. Remove salt solution by aspiration.
- 2) Dispense enough trypsin or trypsin•EDTA solution into culture vessel(s) to completely cover the monolayer of cells and place in  $37\text{ }^{\circ}\text{C}$  incubator for approximately 2 minutes.
- 3) Remove the trypsin or trypsin•EDTA solution by aspiration and return closed culture vessel(s) to incubator. The coated cells are allowed to incubate until cells detach from the surface. Progress can be checked by examination with an inverted microscope.

Note: The time required to remove cells from the culture surface is dependent on cell type, population density, serum concentration in the growth medium, potency of trypsin, and time since last subculture. Trypsin causes cellular damage and time of exposure should be kept to a minimum.

- 4) When trypsinization process is complete the cells will be in suspension and appear rounded.
- 5) It is advisable to add serum or medium containing serum to the cell suspension as soon as possible to inhibit further tryptic activity which may damage cells. Soybean trypsin inhibitor, Catalog Number T9008, can also be added at an equimolar concentration to inhibit the trypsin that is present. Soybean trypsin inhibitor is used when culturing in serum-free conditions.
- 6) Cells can be resuspended by gently pipetting the cell suspension to break up the clumps. Further dilution can be made, if required, for cell counts and/or subculturing.

### References

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