

## Native *Bacillus polymyxa* Neutral Protease (Dispase)

Cat. No. NATE-0482

Lot. No. (See product label)

### Introduction

- Description** Neutral protease (Dispase) is a non-mammalian animal origin free (AOF) metallo, neutral protease. Its mild proteolytic action makes the enzyme especially suitable for the preparation of primary cells and secondary (subcultivation) cell culture, since it is gentle on cell membranes. This protease is also used as a secondary enzyme in cell isolation and tissue dissociation applications, commonly used with collagense.
- Applications** Tissue disaggregation and subcultivation; Prevention of unwanted cell clumping; Preparation of cells for culture; Separation of intact epidermis from dermis and intact epithelial sheet in culture from the substratum (Kurt et al. 1989); Harvest and transfer of normal, diploid cells and cell lines (Matsumura et. al 1975); Gentle and intact detachment of epidermal cells (Kitano and Okada 1983).
- Synonyms** Bacillolysin; EC 3.4.24.28; Bacillus metalloendopeptidase; Bacillus subtilis neutral proteinase; anilozyme P 10; Bacillus metalloproteinase; Bacillus neutral proteinase; megateriopeptidase; Neutral Protease (Dispase)

### Product Information

- Source** Bacillus polymyxa
- Form** lyophilized powder
- EC Number** EC 3.4.24.28
- CAS No.** 9001-92-7
- Molecular Weight** 32.5 kDa
- Purity** Chromatographically purified
- Activity** > 4 units per mg dry weight
- Isoelectric point** 5.14 (Theoretical)
- Optimum pH** 5.9-7.0 (Fogarty and Griffin 1973)
- Composition** The enzyme is known to contain 1g-atom of zinc per g-mol of purified enzyme. If this zinc component is removed by chelating agents such as EDTA or EGTA, an inactive apoenzyme is obtained. Calcium has been detected in the purified protein and is believed to play a role in maintaining the structure and configuration, and preventing autolysis (Griffin and Fogarty 1973 and Alvarez 2006).
- Specificity** Neutral protease is a non-specific metalloprotease. It cleaves fibronectin, collagen IV, and to a lesser extent collagen I, but it does not cleave collagen V or laminin. It hydrolyzes N-terminal peptide bonds of non-polar amino acid residues and may preferentially attack denatured and intercellular proteins with exposed hydrophobic amino acid residues. It is believed to bind one zinc ion and four calcium ions per subunit. Unlike other Bacillus species that produce neutral, alkaline, or a mixture of both proteases, Paenibacillus polymyxa is one of three species that produces only a neutral protease (Fogarty and Griffin 1973).
- Activators** Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, and Al<sup>3+</sup> Manganese has a greater activating effect in the case of

<b>Activators</b>	Cu <sup>2+</sup> , Mg <sup>2+</sup> , Mn <sup>2+</sup> , Fe <sup>2+</sup> , and Al <sup>3+</sup> . Manganese has a greater activating effect in the case of Paenibacillus polymyxa than other Bacilli neutral proteases (Griffin and Fogarty 1973).
<b>Inhibitors</b>	EDTA, EGTA, Hg <sup>2+</sup> ; Other heavy metals (Griffin and Fogarty 1971); Not serum
<b>Unit Definition</b>	One Unit releases one micromole of Folin positive amino acids, measured as tyrosine, at 37°C, pH 7.5, using casein as the substrate.

### ***Storage and Shipping Information***

<b>Storage</b>	Store at 2-8°C
<b>Stability</b>	Stable at 2-8°C for 12 months. Aliquot and Store at -20°C after reconstitution with water or commonly used balanced salt solutions or media.