

## Product Information

# Phosphatase, Alkaline from bovine intestinal mucosa

Ammonium sulfate suspension,  $\geq 2,000$  DEA units/mg protein

**P5521**

## Product Description

CAS Registry Number: 9001-78-9

Enzyme Commission (EC) Number: 3.1.3.1

Synonym: alkaline phosphomonoesterase, phosphomonoesterase, glycerophosphatase, alkaline phosphohydrolase, alkaline phenyl phosphatase, orthophosphoric-monoester phosphohydrolase (alkaline optimum)

$K_M$ :

- $1.5 \times 10^{-3}$  M (*p*-nitrophenyl phosphate)
- $19 \times 10^{-3}$  M (phosphoenolpyruvate)

Molecular mass:<sup>1,2</sup> 140–160 kDa

$E_{278}^{1\%} = 7.6$ –10.5

Isoelectric point:<sup>3-5</sup> several isozymes with a pI range of 4.4–5.8

Bovine intestinal alkaline phosphatase is a dimeric, membrane-derived glycoprotein.<sup>1,2,6</sup> At least three isoforms exist, which typically possess two N-linked and one or more O-linked glycans per monomer.<sup>1</sup> The enzyme requires zinc, and magnesium or calcium divalent ions for activity.<sup>3</sup> The enzyme contains approximately 12% carbohydrate (6% hexoses and 6% other neutral sugars).<sup>2</sup> Each molecule of alkaline phosphatase contains four zinc atoms and four disulfide bridges.<sup>2</sup> Maximal activity with alkaline phosphatase is achieved in the presence of magnesium.<sup>7</sup>

Alkaline phosphatase has a broad specificity for phosphate esters of alcohols, amines, pyrophosphate, and phenols. It is routinely used to dephosphorylate proteins and nucleic acids.<sup>8-10</sup> Other applications of alkaline phosphatase include conjugation to antibodies and other proteins for ELISA, Western blotting, and histochemical detection.<sup>11,12</sup>

Alkaline phosphatase may be used to dephosphorylate the 5'-termini of DNA or RNA to prevent self-ligation. DNA or RNA can also be tagged with radiolabeled phosphate (via T4 polynucleotide kinase) after dephosphorylation with alkaline phosphatase.<sup>13</sup> Alkaline phosphatase has also been used to dephosphorylate casein and other proteins.<sup>14,15</sup>

Several publications,<sup>16,17</sup> theses,<sup>18,19</sup> and dissertations<sup>20-24</sup> have cited use of product P5521 in their research protocols.

### pH optimum

- The enzyme is most stable in the pH range 7.5–9.5.<sup>2</sup>
- The pH optimum for enzymatic activity is pH 8–10.
- The pH optimum will change depending upon substrate, substrate concentration, and ionic concentration.<sup>4</sup>
- The enzyme activity for this product is determined at pH 9.8, using a diethanolamine (DEA) buffer enzyme assay.

### Substrates<sup>9,10,25</sup>

Alkaline phosphatase catalyzes the hydrolysis of phosphate monoesters. Substrates that can be hydrolyzed by alkaline phosphatase include:

- *p*-nitrophenyl phosphate
- Phenyl phosphate
- Phenolphthalein phosphate
- $\alpha$ -glycerol phosphate
- $\beta$ -glycerol phosphate
- 2-phosphorylglycerate, triosephosphate
- Glucose 6-phosphate

- Glucose 1-phosphate
- Fructose 1-phosphate
- Fructose 6-phosphate
- Adenosine 5-phosphate
- Adenosine 3-phosphate
- Phosphoenolpyruvate
- $\beta$ -nicotinamide adenine dinucleotide phosphate

### Inhibitors<sup>5,10</sup>

- Chelating agents
- Arsenate
- Cysteine
- Iodine
- Inorganic phosphate
- Pyrophosphate
- Diisopropyl phosphate
- Triphenylphosphate
- Diisopropyl fluorophosphate
- L-phenylalanine

Levamisole (such as Cat. No. L9756) is typically used to inhibit endogenous alkaline phosphatase activity, while only slightly inhibiting the intestinal enzyme.<sup>26,27</sup>

### Precautions and Disclaimer

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.

### Product

This product is supplied as an ammonium sulfate suspension, in 3.2 M ammonium sulfate containing 1 mM magnesium chloride ( $\text{MgCl}_2$ ) and 0.1 mM zinc chloride ( $\text{ZnCl}_2$ ), pH 7.0.

Specific Activity:  $\geq 2,000$  DEA units/mg protein

Unit Definition: One DEA unit will hydrolyze 1  $\mu\text{mole}$  of 4-nitrophenyl phosphate per minute at pH 9.8 at 37 °C. Diethanolamine (DEA) units are measured in a 1.0 M DEA buffer (pH 9.8) containing 0.5 mM  $\text{MgCl}_2$ , with a substrate concentration of 15 mM.

### Storage/Stability

Store the product, as supplied, at 2-8 °C. **Do not freeze ammonium sulfate suspensions.**

### Preparation Instructions

Dilute solutions of alkaline phosphatase should be prepared in 10 mM Tris HCl (pH 8.0), 1-5 mM  $\text{MgCl}_2$ , and 0.1-0.2 mM  $\text{ZnCl}_2$ . 50% glycerol can be included for long term storage at 2-8 °C.<sup>12</sup>

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