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Product data sheet

**Alkaline Phosphatase (AP) (Conjugation/EIA Grade)**

**Cat# EW-ALP15-N-1, Size: 1 mg; Cat# EW-ALP15-N-5, Size: 5 mg; Cat# EW-ALP15-N-10, Size: 10 mg**  
**Form: powder Storage: Store at -20°C**

**Description**

Alkaline phosphatases (APs) are highly ubiquitous enzymes, present in all species from bacteria to man. In humans, APs are encoded by a multi-gene family composed of four loci; i.e., tissue-nonspecific AP, also called bone/liver/kidney AP, (Weiss et al. J. Biol. Chem., 264, 12002-12010, 1988), intestinal (Henthorn et. al., J.Biol. Chem., 263, 12020-12027, 1988). The sequence and complexity of the AP genes from other vertebrates and lower species are now being elucidated. The biological function of AP isozymes is still unknown. In vitro, the enzymes behave as phosphotransferases at neutral pH. The use of phosphate acceptor molecules ( diethanolamine, tris, 2-amino-2-methyl-1-propanol) in the buffered substrate solutions increases the reaction rates and, thus, the sensitivity of assays based on AP determinations.

ALP is commonly used as a label in immunoassays such as ELISA, and in blotting and histochemistry. Once conjugated to antibodies, antigens, or streptavidin, its low backgrounds and linear reaction rate enables increased sensitivity over extended incubation times. It can be used with a variety of substrates producing precipitated or soluble chromogens, or with chemiluminescent substrates for enhanced sensitivity.

**Form and Storage**

**Form:** Freeze-dried powder  
**Solubility:** Distilled water or dilute buffer  
**Stability:** Store at -20° C (-4° F)  
**Activity:** 3,000-6,000 U/mg protein

The powdered AP should be stored in the freezer (-20 °C). If properly stored, these products have a shelf life of at least two years. Solutions lose <2 % of their activity per week if stored at -20 °C.

**PRODUCT SPECIFICATION-Unit Definitions**

*Glycine Units:*

That amount of enzyme causing the hydrolysis of one micromole of p-Nitrophenyl phosphate per minute at pH 9.6 and 25°C (glycine buffer).

*DEA Units :*

The amount of enzyme causing the hydrolysis of one micromole of p-Nitrophenyl phosphate per minute at pH 9.8 and 37°C (diethanolamine buffer).

*Unit Conversion:*

One Glycine unit as described above is equivalent to approximately three DEA units at pH 9.8 and 37°C.

**Assay Methods**

The increase in absorbance at Hg 405 nm is measured.

**Reagents**

1. Diethanolamine buffer (1 mol/L; pH 9.8; MgCl<sub>2</sub> 0.5 mmol/L): Dilute 10.6 grams (9.7 mLs) diethanolamine (99%) with

distilled H<sub>2</sub>O, add 0.05 ml MgCl<sub>2</sub> solution (2) and adjust the pH to 9.8 (at 37 °C) with HCl, >= 2mol/L, adjust to 100 ml with distilled H<sub>2</sub>O.

2. Magnesium chloride solution (1 mol/L): Dissolve 20.3g MgCl<sub>2</sub>•6 H<sub>2</sub>O in 100 ml distilled H<sub>2</sub>O
3. 4-Nitrophenyl phosphate solution (0.67 mol/L): Weigh 250 mg 4-nitrophenyl phosphate, Na salt add 1.0 ml distilled H<sub>2</sub>O to dissolve.
4. Diluent (0.1 mol/L; pH 7.6): Dissolve 1.86g TEA •HCl in distilled H<sub>2</sub>O, add 0.1 ml MgCl<sub>2</sub> solution (2) and 0.1 ml ZnCl<sub>2</sub> (0.1 mol/L; prepare freshly); adjust the pH value to 7.6 with NaOH, 1 mol/L and adjust to 100 ml with distilled H<sub>2</sub>O.
5. Sample solution: Dilute enzyme solution to an activity of 0.05 to 0.06 U/ml with buffer (4). Usual final dilutions will be in the 1:200,000 to 1:600,000 range. Let stand for approximately 15-20 min. at room temperature before conducting assay

**Procedure**

1. Set spectrophotometer(with temperature control) at 405 nm and 37°C.
2. Into a cuvette pipette the following:

Buffer(1),	2.90 ml
4-nitrophenyl phosphate	0.05 ml
3. Incubate cuvette in spectrophotometer at 37°C for 6-8 minutes to achieve temperature equilibration and establish blank rate, if any.
4. Add 0.1 ml of diluted enzyme to the cuvette, mix and record increase in absorbance at 405 nm for 5 minutes.
5. Calculate (ΔE405nm/min) from the linear portion of the curve.

For in vitro research use only

**Related Material available for GSI**

Single solution, ready to use, TMB substrates for Blotting & ELISA

Anti-Rabbit HRP conjugates; Anti-Mouse, human, rat, and Monkey IgG-HRP and subsotype specific conjugates

**Western blot recycling kit** (Use the same blot/strip to probe with multiple antibodies,

Chemiluminescence Substrates and Western blot kits (save by complete kit that includes Anti-rabbit IgG-HRP conjugate, blocking buffer, wash buffer, and Chemiluminescence Substrates for processing 15-30 std size blots).

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