ALPHA DIAGNOSTIC INTERNATIONAL

Product Data Sheet

Alkaline Phosphatase (AP) (Conjugation/EIA Grade)

Cat# ALP15-N-1, Size: 1 mg; **Cat# ALP15-N-5, Size:** 5 mg; **Cat# ALP15-N-10, Size:**10 mg

Form: powder Storage: Store at -20oC

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
Mol wt	~140 Kda

The powdered AP should be stored in the freezer (–0 °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 25oC (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 37oC (diethanolamine buffer).

Unit Conversion:
One Glycine unit as described above is equivalent to approximately three DEA units at pH 9.8 and 37oC.

Assay Methods
The increase in absorbance at Hg 405 nm is measured.

Reagents

1. Diethanolamine buffer (1 mol/L; pH 9.8; $MgCl_2$ 0.5 mmol/L): Dilute 10.6 grams (9.7 mLs) diethanolamine (99%) with distilled H_2O, add 0.05 ml $MgCl_2$ solution (2) and adjust the pH to 9.8 (at 37°C) with HCl, >= 2mol/L, adjust to 100 ml with distilled H_2O.

2. Magnesium chloride solution (1 mol/L):
Dissolve 20.3g $MgCl_2$•6 H_2O in 100 ml distilled H_2O

3. 4-Nitrophenyl phosphate solution (0.67 mol/L):
Weigh 250 mg 4-nitrophenyl phosphate, Na salt add 1.0 ml distilled H_2O to dissolve.

4. Diluent (0.1 mol./L; pH 7.6):
Dissolve 1.86g TEA •HCl in distilled H_2O, add 0.1 ml $MgCl_2$ solution (2) and 0.1 ml $ZnCl_2$ (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_2O.

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 ADI

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

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