

**ENZYMES**  
CAT# ALKP-70-6471  
(15 mg vial, Bulk)  
EC# 3.1.3.1

## ENZYMES

# Smartbond Alkaline Phosphatase

ORIGIN *Microbial*

CAT# ALKP-70-6471  
EC# 3.1.3.1

### ► SPECIFICATIONS

|                              |                                                                                    |
|------------------------------|------------------------------------------------------------------------------------|
| <b>Appearance</b>            | Clear to slightly opaque solution                                                  |
| <b>Specific Activity</b>     | ≥ 15,000 U/mg at 37°C                                                              |
| <b>Protein Concentration</b> | 15.0 ± 5.0 mg/ml                                                                   |
| <b>Formulation</b>           | 3 M NaCl, Triethanolamine, plus Mg <sup>2+</sup> and Zn <sup>2+</sup> ions, pH 7.5 |
| <b>Stability</b>             | +2 to +8°C for 12 months                                                           |

### ► PRODUCT FORMAT

Ready-to-use/liquid formulation. Available in 15 mg vial or 500 mg container (bulk).  
A 15 mg vial contains ≥ 225 KU.

### ► APPLICATION

Smartbond Alkaline Phosphatase is a recombinant highly active product. It is an alternative to conventional Alkaline Phosphatase products made from bovine intestine.

Alkaline Phosphatase is commonly used as a labelling enzyme in immunoassays such as ELISA. Smartbond Alkaline Phosphatase can be used with commonly used substrates including pNPP, BCIP, MUP and CSPD. Effective conjugation of Smartbond Alkaline Phosphatase can be achieved via its accessible amino groups; it does not contain carbohydrate groups.

### ► UNIT DEFINITION

One unit is defined as the amount of Alkaline Phosphatase that can hydrolyse 1.0 μmole pNitrophenyl Phosphate per minute at pH 9.8 (Diethanolamine + MgCl<sub>2</sub>) at 37°C.

### ► ASSAY PRINCIPLE

Alkaline Phosphatase catalyses the hydrolysis of p-Nitrophenyl Phosphate to liberate p-Nitrophenol which is yellow and can be measured spectrophotometrically at 405 nm.



Abbreviations: pNPP, p-nitrophenyl phosphate; BCIP, 5-bromo-4-chloro-3-indolyl phosphate p-toluidine salt; MUP, 4-methylumbelliferyl phosphate; CSPD, disodium 3-(4-methoxyspiro {1,2-dioxetane-3,2'-(5'-chloro)tricyclo {3.3.1.1<sup>3,7</sup>} decan}-4-yl)phenyl phosphate.

**SEKISUI**  
DIAGNOSTICS

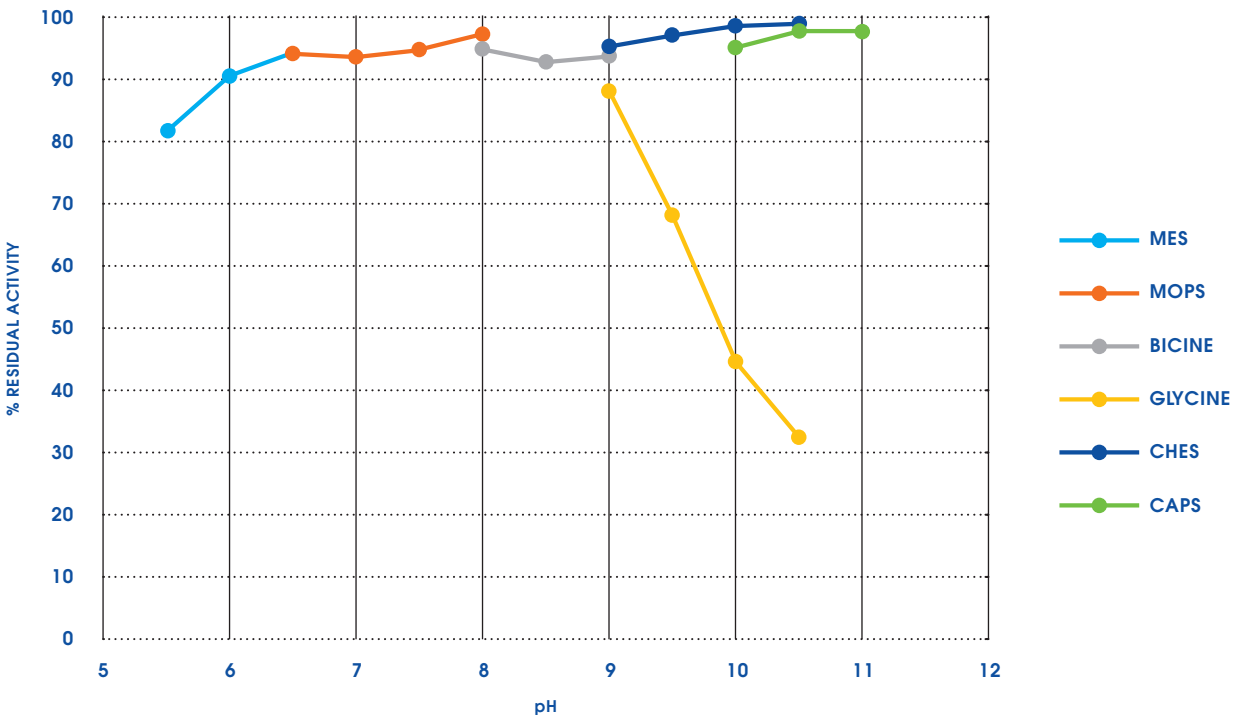
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**CHARACTERISTICS**

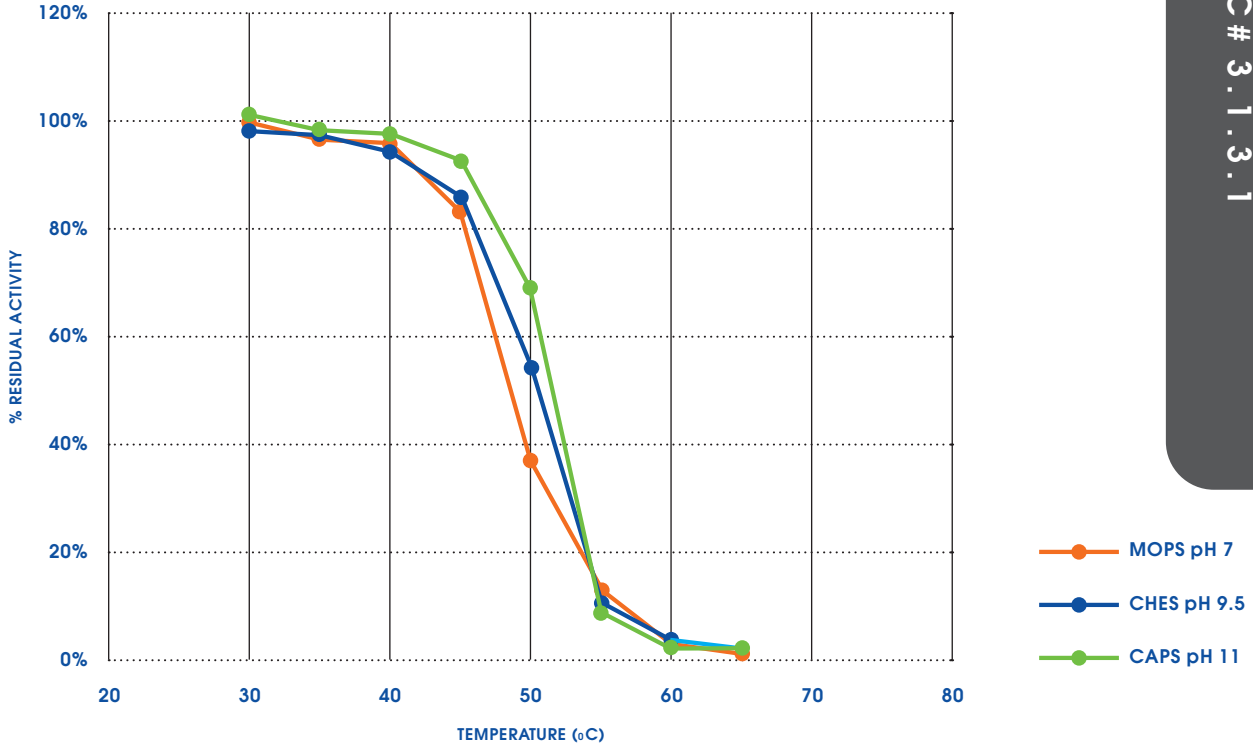
|                                 |                                                                                                                                                                                                                                    |
|---------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Molecular Weight:</b>        | ~55 kD (subunit)                                                                                                                                                                                                                   |
| <b>Isoelectric Point:</b>       | ~4.3                                                                                                                                                                                                                               |
| <b>Michaelis Constant (Km):</b> | 3 mM (p-Nitrophenyl phosphate)                                                                                                                                                                                                     |
| <b>Optimum pH:</b>              | Highest activity recorded at pH 11.0<br><i>(Results based on pNPP assay with 0.05 M buffer pH 7.5 to 11.0, containing 0.5 mM MgCl<sub>2</sub>)</i>                                                                                 |
| <b>Optimum Temperature:</b>     | 45°C<br><i>(Results based on pNPP assay with 0.05 M CAPS buffer pH 11.0, containing 0.5 mM MgCl<sub>2</sub>)</i>                                                                                                                   |
| <b>pH Stability:</b>            | pH 6.0 to 11.0 excluding Glycine effect*<br><i>(Results derived after incubation of the enzyme for 20 hr at 25°C in 0.1 M buffer, pH 5.5 to 11.0, containing 0.5 mM MgCl<sub>2</sub>)</i>                                          |
| <b>Thermal Stability:</b>       | Stable up to 40°C at pH 9.5 or up to 45°C at pH 11.0<br><i>(Results based on incubating enzyme for 15 min in 0.1 M buffer at pH 7, 9.5 or 11.0 containing 0.5 mM MgCl<sub>2</sub> at temperatures within the range 30 to 65°C)</i> |
| <b>Inhibitors:</b>              | Hg <sup>2+</sup> , Cu <sup>2+</sup> , Co <sup>2+</sup> , Zn <sup>2+</sup> , Ni <sup>2+</sup> , Pb <sup>2+</sup> , Fe <sup>2+</sup> , EDTA (Table 1)                                                                                |

\*Glycine significantly impacts the activity of Smartbond Alkaline Phosphatase at elevated concentration (Table 2) and also destabilises the product above pH 9.0. The inhibitory effect of Glycine toward alkaline phosphatase has been known for some time (Ref: G.E. Delory and E. J. King, *Biochemistry Journal*, vol. 39, no. 3, pp. 245-245, 1945).

**pH STABILITY**



**THERMAL STABILITY**



**TABLE 1: ACTIVATORS AND INHIBITORS**

*(The effect was evaluated following incubation of Smartbond Alkaline Phosphatase for 1 hour at 25°C in the presence of each chemical listed at the specified concentration)*

| CHEMICAL          | CONCENTRATION | RESIDUAL ACTIVITY | CHEMICAL             | CONCENTRATION | RESIDUAL ACTIVITY |
|-------------------|---------------|-------------------|----------------------|---------------|-------------------|
| Tween 20          | 0.10 %        | 117%              | DTT                  | 1 mM          | 79%               |
| Brij 35           | 0.10 %        | 115%              | SDS                  | 0.10%         | 68%               |
| Triton X-100      | 0.10 %        | 115%              | MnCl <sub>2</sub>    | 2 mM          | 66%               |
| Span 20           | 0.10 %        | 112%              | FeCl <sub>2</sub>    | 2 mM          | 43%               |
| MgCl <sub>2</sub> | 2 mM          | 104%              | Pb(OAc) <sub>2</sub> | 2 mM          | 38%               |
| CaCl <sub>2</sub> | 2 mM          | 101%              | EDTA                 | 5 mM          | 38%               |
| Na cholate        | 0.10 %        | 98%               | NiCl <sub>2</sub>    | 2 mM          | 17%               |
| NaN <sub>3</sub>  | 20 mM         | 97%               | ZnCl <sub>2</sub>    | 2 mM          | 6%                |
| NaF               | 2 mM          | 96%               | CoCl <sub>2</sub>    | 2 mM          | 5%                |
| Iodacetamide      | 2 mM          | 96%               | CuSO <sub>4</sub>    | 2 mM          | 4%                |
| Levamisole        | 1 mM          | 95%               | HgCl <sub>2</sub>    | 2 mM          | 3%                |
| AgNO <sub>3</sub> | 2 mM          | 83%               |                      |               |                   |

**TABLE 2: EFFECT OF BUFFER TYPE**

*(Results derived using pNPP assay with 1 M buffer at pH 9.8 containing 0.5 mM MgCl<sub>2</sub>)*

| BUFFER         | RELATIVE ACTIVITY |
|----------------|-------------------|
| Diethanolamine | 100%              |
| Tris           | 38%               |
| Glycine        | 0%                |

**THE AMERICAS**

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