

Smartbond Alkaline Phosphatase

Catalogue No: ALKP-70-6471 [15 mg vial or 500 mg container (bulk)]

E.C. Number: 3.1.3.1

1.0 Applications

Smartbond Alkaline Phosphatase is a highly active recombinant product. It is an alternative to conventional Alkaline Phosphatase products made from bovine intestine. It is intended for in vitro immuno-detection methods and not for in vivo use.

2.0 Handling Instructions

2.1 Safety

Refer to the MSDS for safety instruction. Extra care should be taken when removing the crimp to avoid sharps injury.

2.2 Handling

Vials are filled according to mg of Alkaline Phosphatase protein. Invert gently prior to opening the vial and dispensing. The product may appear opaque - this is normal and should not affect performance.

2.3 Use

2.3.1 Conjugation

The product has no glycosylated residues. Effective conjugation can be achieved using linker molecules by attachment to either its His-tag or amino groups.

2.3.2 Chromogenic and Fluorescence based Assay Methods

Smartbond Alkaline Phosphatase can be used with pNPP & MUP substrates.

2.3.2.1 Buffer Selection

For optimal performance, Diethanolamine buffer (50 to 250 mM at pH 10) is recommended containing MgCl₂ (10 mM).

As a precautionary note, assays based on Glycine buffer will give significantly lower values in comparison to Diethanolamine buffer due to the inhibitory/destabilisation effect of Glycine (see Smartbond Alkaline Phosphatase datasheet).

2.3.3 Chemiluminescence based Assay Methods

Smartbond Alkaline Phosphatase can be used effectively with 1,2-dioxetane substrates including AMPPD, CDP-Star and CSPD following the guidelines below:

2.3.3.1 Buffer Selection

Buffer and pH should be optimised for the substrate used. As a guide:

Substrate	Recommended Buffer	Recommended pH Range for Assay
AMPPD	Diethanolamine	9.5-9.8 [†]
CSPD	Diethanolamine	9.5-9.8 [†]
CDP-Star	Tris	8.5-8.8 [†]

[†]Based on pH measurement at 25°C.

2.3.3.2 Enhancer Molecules

The inclusion of chemiluminescence enhancers are recommended for optimal performance. The presence of BSA can significantly impact the chemiluminescence signal if used in combination with standard enhancer molecules. Recommended conditions for using either Emerald-II™ or Sapphire-II™ (based on 0.25 mM substrate concentration) are as follows:

Substrate	Emerald-II™	Sapphire-II™
AMPPD	200 mM DEA pH 9.8 0.5 mM MgCl ₂ 2X Enhancer*	200 mM DEA pH 9.8 50 mM MgCl ₂ 2X Enhancer*
CSPD	200 mM DEA pH 9.8 1 mM MgCl ₂ 2X Enhancer*	200 mM DEA pH 9.8 100 mM MgCl ₂ 2X Enhancer* 1 M KCl
CDP-Star	200 mM Tris pH 8.8 1 mM MgCl ₂ 0.5X Enhancer*	200 mM Tris pH 8.8 100 mM MgCl ₂ 2X Enhancer* 1 M KCl

*Based on Enhancer solution supplied as 10X concentrate.

2.3.3.3 Commercially Available (Chemiluminescence) Reagent Kits

Commercially available (chemiluminescence) reagent kits can be utilised but are sub-optimal for Smartbond Alkaline Phosphatase. In such cases, inclusion of or dilution with BSA (up to 0.1 %), pure water (up to 50%), or KCl (up to 2 M) may help increase the signal.

2.4 Storage and Shelf Life

12 months shelf life. Vials should be stored at +2 to +8 °C.

Effective Date: 6th September 2018

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