

## APPLICATION NOTE

# Purification of Alkaline Phosphatase from Calf Mucosal Extract using Mimetic Blue AP A6XL

### Introduction

*Mimetic' Blue AP A6XL is a synthetic affinity absorbent developed specifically for the purification of alkaline phosphatase from calf intestinal mucosa and other sources*

### Experimental Conditions

Extract (17 ml in 10 mM Tricine buffer, pH 8.5) was applied to a 2 ml column of Mimetic' Blue AP A6XL at a flow rate of 0.5ml/min. Elution was undertaken using 5 mM phosphate.

### Results

Alkaline phosphatase bound to the Mimetic Blue AP A6XL column and was eluted using 5 mM phosphate (Fig 1).

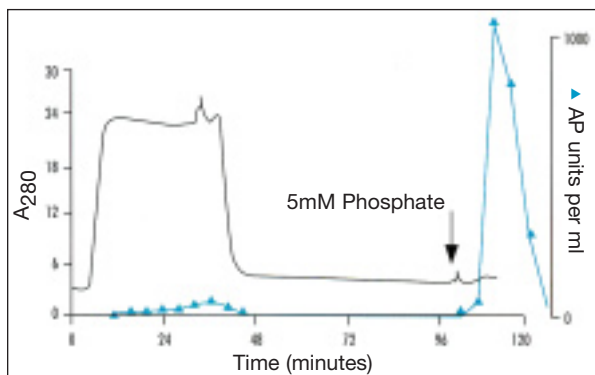


Fig 1. FPLC trace of the purification of alkaline phosphatase from a calf mucosal extract using MIMETIC' Blue AP A6XL.

Results indicated a 170-fold increase in specific activity with 86% of the enzyme recovered in the elution peak. Alkaline phosphatase activity was measured at 25°C using p-nitro-phenolphosphate as substrate and 25 mM glycine buffer, pH 9.6. Calf intestinal mucosa from milk fed calves was obtained from Grampian Enzymes, Ltd, Aberdeen, Scotland.

### Affinity chromatography of commercial 'highly pure' alkaline phosphatase

Two ml of a 1 mg/ml 'high purity' alkaline phosphatase were applied to a 2ml column under the same conditions as described above. The detector response indicates the removal of A280 absorbing contaminants from the dialysed high purity enzyme preparation (Fig 2).

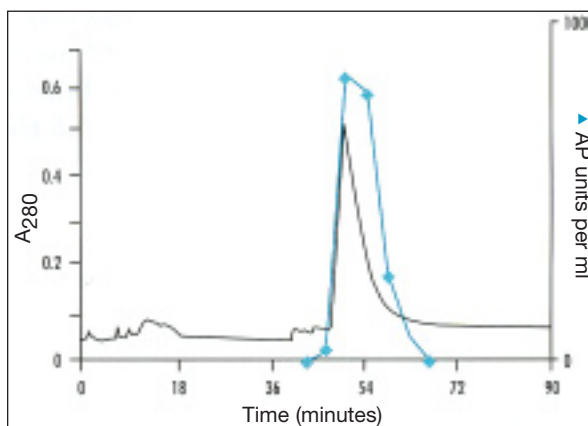


Fig 2. Affinity chromatography of 'highly pure' calf intestinal alkaline phosphatase on MIMETIC' Blue AP A6XL.

### References

Linder, N.M, Jeffcoat, R. & Lowe, C.R., J Chromatography, 473, 227-240 (1989).

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