

## APPLICATION NOTE

# Development of a Purification Strategy for Recombinant Interferon-alpha-2b

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### Introduction

Interferons (IFNs) are a multigene family of proteins exhibiting a broad range of biological properties. A two step purification procedure involving the use of a Mimetic Blue 1 P6XL affinity chromatography resin for the purification of Interferon-alpha-2b (IFN- $\alpha$ 2b) is outlined below.

### Experimental Conditions

The starting material was cell culture supernatant from a HEK-293 cell line expressing recombinant IFN- $\alpha$ 2b at a titre of 10  $\mu$ g/mL. Clarified feedstocks were concentrated to 100  $\mu$ g/ml for use in experiments.

Initial capture of the IFN- $\alpha$ 2b was undertaken using a Fractogel<sup>®</sup> EDM COO (M) ion exchange column (EM Industries, Germany). This choice came from the procedure developed by Beldarrain et al (2001) where it was used as a final polishing step in the purification of human IFN- $\alpha$ 2b. The column was equilibrated with 50 mM ammonium acetate buffer, pH 5.0 at a flow rate of 50 cm/hr<sup>-1</sup> (0.65 mL/min). For elution a buffer of 50 mM ammonium acetate/0.1 M NaCl, pH 5.0 was used.

The elution fraction from the Fractogel ion-exchange column was concentrated and diafiltered against 25 mM sodium phosphate buffer, pH 7.0, using a 10,000 NMWCO centrifugal ultrafiltration device.

The sample was loaded directly onto a Mimetic Blue 1 P6XL affinity chromatography column (ProMetic BioSciences Ltd., UK) equilibrated with 25 mM sodium phosphate buffer, pH 7.0. Elution was undertaken using 25 mM sodium phosphate/0.5 M NaCl buffer, pH 7.0.

SDS-PAGE analysis of the starting material and purified fractions was performed under reducing conditions on a 12% (w/v) polyacrylamide Novex gel. Visualization of the bands was achieved using Coomassie Blue stain. Western blot analysis was

undertaken using an anti-IFN- $\alpha$ 2b polyclonal antibody raised in rabbit (1:1000) and a goat anti-rabbit HRP conjugate (1:1000).

### Results and Conclusions

IFN- $\alpha$ 2b bound to the Fractogel<sup>®</sup> EDM COO ion exchanger at pH 5.0 and was eluted using 0.1M NaCl (Figure 1, below). The elution fraction was purified further using the Mimetic Blue 1 affinity chromatography column. Elution was undertaken using 0.5 M NaCl (Figure 1, below).



Figure 1. SDS-PAGE gel (left) and Western blot (right) analysis of the load and elution fractions collected for the two-step capture and purification procedure developed for IFN- $\alpha$ 2b.

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The SDS-PAGE and Western blot analysis showed that IFN- $\alpha$ 2b is present in the load (Lane 1), Fractogel elution (Lane 3), and Mimetic Blue 1 P6XL elution (Lane 4). An IFN- $\alpha$ 2b standard was run for comparison (Lane 2). The high molecular weight band seen in Lanes 1, 3, and 4 is probably an aggregate, as shown by interaction with the antibody.

The method shown here clearly demonstrates that affinity chromatography can be successfully applied to the purification of IFN- $\alpha$ 2b.

*For more information*

Beldarrain A; et al. Biotechnol. Appl. Biochem. (2001) 33, 173–182.

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