entropix

Enrichment of Recombinant Therapeutic Proteins Using SCOPE™ Fractionation

Entropix and Daresbury Proteins Ltd

Daresbury Proteins

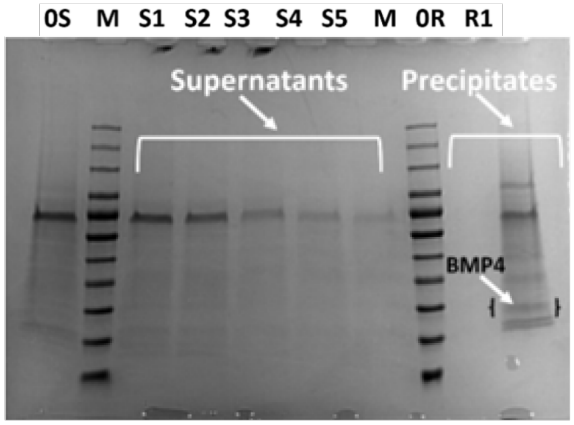
INTRODUCTION

Daresbury Proteins Ltd specializes in the development and manufacture of complex recombinant proteins for a range of therapeutic and research applications expressed in mammalian cell lines. Some target proteins are secreted at low levels into the cell culture medium making it challenging to purify them away from contaminants. Entropix has developed SCOPE™, a groundbreaking protein fractionation technology that preserves the integrity and functionality of proteins. By adding increasing amounts of the SCOPE™ reagent, proteins can be captured from solution into a solid precipitate; after precipitation each fraction can be recovered by re-solvation using RESOLVE™. Entropix and Daresbury Proteins have collaborated to investigate the potential for enrichment of recombinant growth factors and cytokines, exemplified here by the Bone Morphogenetic Protein, BMP-4, using the newly developed SCOPE™ and RESOLVE™ reagents. This enrichment method can simplify the manufacturing process by acting as a rapid pre-purification step prior to the usual chromatography process while fully preserving authentic protein activity.

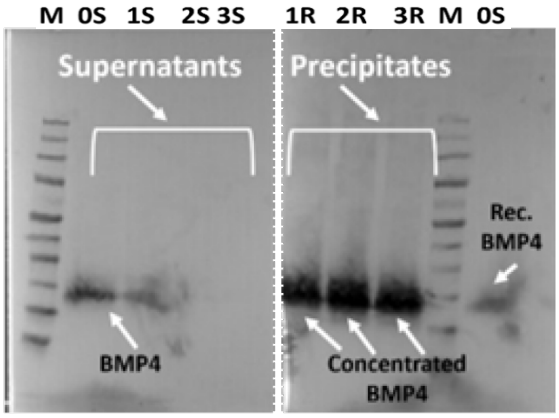
EXPERIMENT & RESULTS

The cell expression medium containing recombinant, untagged BMP4 was successfully fractionated by the addition of a range of SCOPE™ concentrations. The SDS gel below left, shows the depletion of supernatant proteins following addition of increasing concentrations of SCOPE™ (lanes S1-S5) to the cell medium (lane 0S). The precipitates formed by SCOPE addition can be re-solvated by the addition of RESOLVE™ as shown by comparing lanes 0R (no RESOLVE™) with lane R1 (excess RESOLVE™). The loss of BMP4 from the cell medium is confirmed using a BMP4 specific antibody in a Western Blot of SDS PAGE separated fractions (lanes 2S and 3S show that BMP4 has been precipitated from the medium), while lanes 1R-3R show that BMP4 present in the precipitates, can be fully recovered in a fraction of the volume using RESOLVE at several concentrations. SCOPE™ and RESOLVE™ provide a rapid, simple, cost-effective method for the enrichment of proteins in complex mixtures. The companies are extending their collaboration to incorporate the SCOPE™ process in regular production.

SDS PAGE gel analysis of fractionated medium



Western Blot analysis of fractions



Dr Jennet Beasley
Founder and director of Daresbury Proteins:

"As someone who has always worked with complex secreted recombinant proteins requiring extensive processing for chromatography purification, I really appreciate the value and simplicity of the SCOPE-RESOLVE™ technology in our applications. It can open up entirely new opportunities for protein purification".

Professor David Hornby
CSO and co-founder of Entropix:

"This was a text-book outcome! We have demonstrated the value of SCOPE™ for the rapid enrichment of a valuable recombinant protein from a complex mixture of contaminants. This reinforces our earlier work using SCOPE™ to simplify cellular proteomes as a powerful method for studying protein interactions and complexes using mass spectrometry".

For further information, samples or trials please contact enquiries@entropix.co.uk