Expedeon Refold Master

Case Study – Interleukin 15

Introduction

IL-15 is a cytokine involved in several steps of the inflammation cycle. It is produced by several cells including macrophages and stimulates T-lymphocyte and NK cell proliferation making it an interesting disease target. The molecule is composed of 144 AA and weighs 12.7KDa with a pI of 4.5. Its structure contains 2 S-S bonds and consists of 4α-helix bundles.

Summary

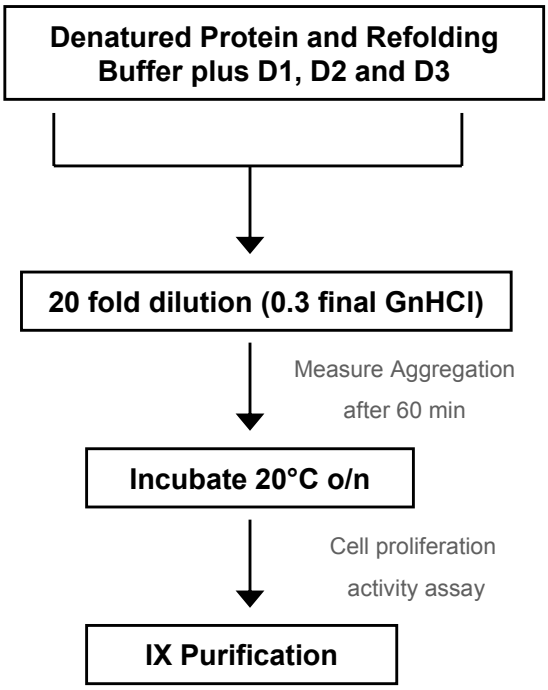
Expedeon's protective agents, NV10, NV20 and NV30 all significantly improved refolding of active wild –type (WT) and derivitised (DV) IL-15. NV10 was most effective at suppressing aggregation, by 92% and 93% for WT and DV IL-15 respectively. Refold yields increased significantly from <1% to >75% for both NV10 and NV20 and there was a corresponding 3 log improvement in activity for both the WT and DV forms.

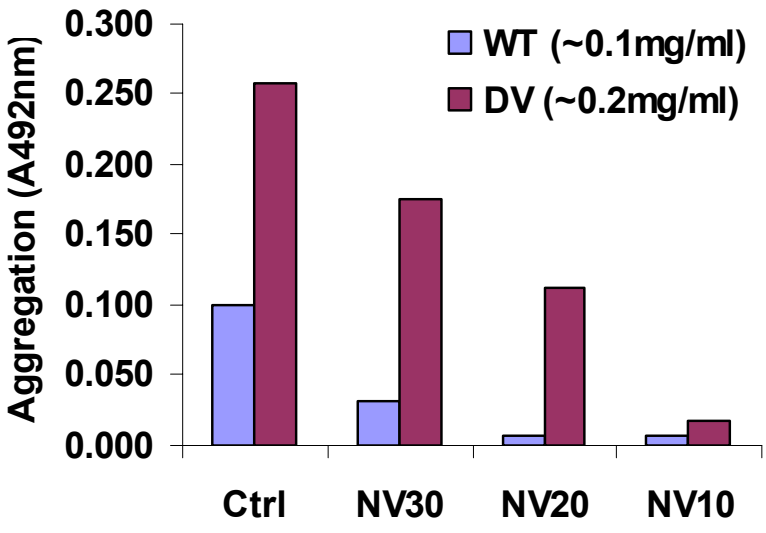
expedeon
PROTEIN SOLUTIONS

Expedeon has developed a novel, proprietary technology that is radically improving the processing of proteins. Our NVoy Polymer technology provides a simple method that eliminates the need to screen multiple conditions whilst reducing yield losses due to aggregation and allowing higher protein concentrations to be analysed. It's generic use allows a Yes/No answer to be reached quickly on refolding success and the technology is scaleable for low cost processing.

Refold Methodology

Inclusion bodies were prepared using a 6M GnHCl, 32mM DTT, pH 8.0 denaturation buffer. Denatured protein at 1.8mg/ml and 3.9mg/ml was then refolded using a 50mM Tris buffer, pH 8.0, 5mM GSSG and 3 formulations of Expedeon's protective agent. NV10, NV20 and NV30 denote three different strength protectants that are used to suppress aggregation of protein and replace commonly used additives such as arginine and detergents. The controlled release of protectants allows protein to refold correctly – obvious differences were observed when the strength was varied with maximum aggregation suppression occurring with NV10. Aggregation was reduced by 92.5% and 93.4% for WT and DV respectively.



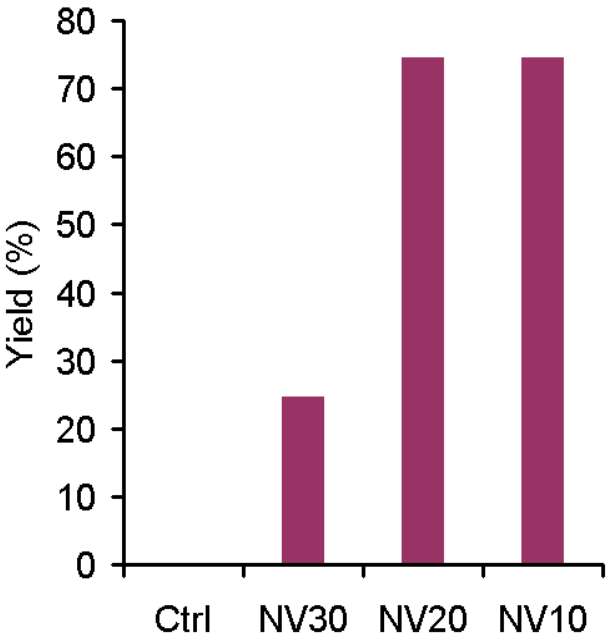


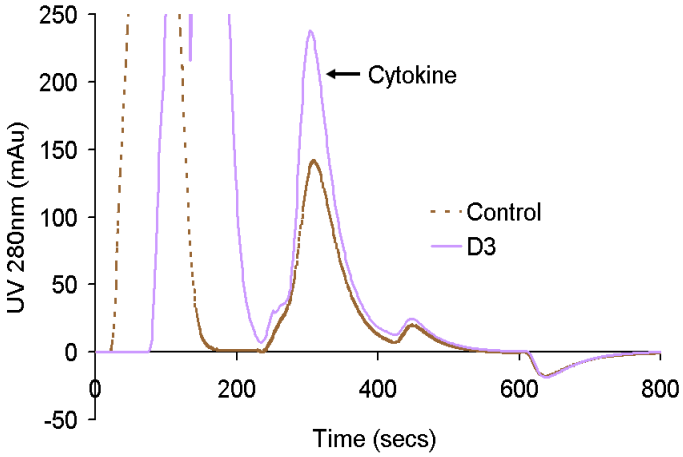
Expedeon Refold Master

Results

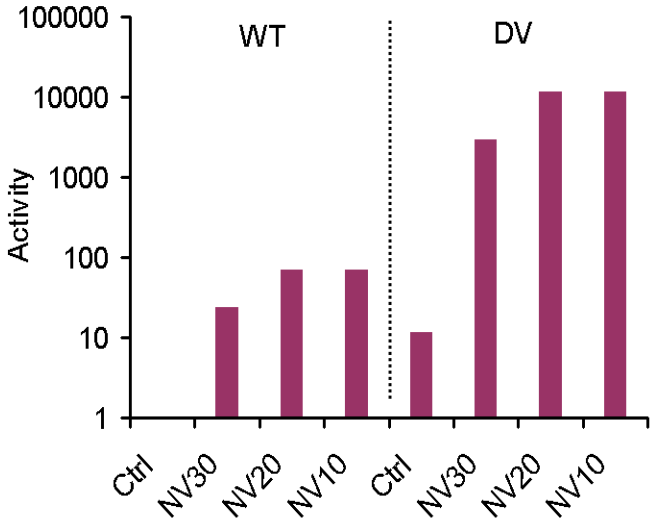
Expedeon's Refold Master improved refolding yields significantly. No yield was detected in control samples (refold buffer without Expedeon's protective additive) but when using both NV10 and NV20 the yield increased to ~75%.

Each sample was purified using a 1ml HiTrap Column of SP Sepharose which was optimised for the control and due to high amounts of protein in all but the NV30 sample the column was overloaded.





As expected after the initial experiments, activity assays showed NV10 and NV20 samples having the highest specific activity. Activity was measured using a cell proliferation assay and the activity was higher in the DV IL-15 than in the WT samples. In both cases the control samples had low or negligible activity. A 3 log improvement in performance was observed upon the use of NV10 for both forms of IL-15, the specific activity again being higher for the DV molecule which showed a >1,000 fold improvement in activity.



Conclusion

Expedeon's Refold Master, incorporating NVoy Polymer technology, is a simple to use kit that can be used to generate excellent refolding yields without the need to screen multiple parameters to find unique conditions for each protein. Since the reagents are compatible with the commonly used analytical methods, downstream analysis was simplified and the technology will allow a seamless transfer from research to production due to the ease of scale up.

References

Thanks go to Dr Nico Mertens of VIB, Belgium for his kind permission to use this data.

Expedeon Ltd, Unit 1A, Button End, Harston, Cambs., CB22 7GX, U.K.
Tel. +44 (0) 1223 873364 • Fax +44 (0) 1223 873371 • E-mail: info@expedeon.com