

NVoy Tech Note

Fusion Protocol

Release 1. © EXPEDION. January 2018

INTRODUCTION

NVoy technology is a quantum leap in protein processing, production and analysis. It uses proprietary NV polymers to enhance protein solubility and stability through the formation of multi-point reversible complexes with proteins without altering their structure.

NVOY POLYMER, NV10, STABILISES FUSION PROTEINS AFTER TAG CLEAVAGE

The expression of recombinant proteins with fusion partners can result in enhanced expression yields, increased solubility and an improved purification route. Subsequent site-specific proteolysis is often required to remove these fusion partners after purification in order to produce native protein. However, cleavage of fusion tags which have been added to enhance solubility frequently results in protein aggregation and loss of yield. The presence of NV10 in cleavage buffers greatly improves protein solubility while maintaining high cleavage efficiency. NV10 can be readily removed from cleaved protein solutions by ion exchange or affinity chromatography, but its compatibility with many downstream applications often allows the user to retain NV10 in solution with target protein, maintaining protein solubility and stability.

PROTOCOL

Aggregation and stability are very protein specific, but a general protocol is given below.

1. Determine the starting protein concentration (using eg. Expedeon's BradfordUltra assay, BCA assay, absorbance at 280nm).
2. Typically, a fivefold excess, by mass, of NV10 will protect the target fusion protein. For example, use 5 mg/ml NV10 for 1 mg/ml protein.
3. Each Stabil-P.A.C. tube contains 10mg NV10 as a lyophilised powder (40mg per tube in a Stabil-PAC MAXI).
4. Add the protein solution to NV10 in Stabil-P.A.C. tubes to get the desired concentration, or make up a stock solution (e.g. 5 mg/ml NV10) by adding buffer or distilled water to each Stabil-P.A.C. tube and then add this stock to the protein cleavage solution before adding the protease.
5. Alternatively, make up the cleavage buffer with the desired NV10 concentration, and use PD10 desalting columns to buffer exchange the target fusion protein into cleavage buffer containing NV10.
6. Continue with tag cleavage according to the standard protocol.
7. NV10 associates with the protein in solution and protects the cleaved native protein from aggregation and instability.
8. NV10 stock solutions (up to 10 mg/ml) can be stored for up to 1 week at 4°C or for longer term at -20°C. More concentrated stock solutions should be used immediately.

TROUBLESHOOTING

- If the protein shows signs of aggregation or heavy losses on cleavage then the relative NV10 concentration can be increased, ie increase NV10 concentration and / or reduce protein concentration.
- Alternatively, a lower NV10 to protein ratio can be used with proteins that have no history of aggregation.

EXAMPLE

A kinase protein was prepared with maltose binding protein as a fusion partner (k-MBP) to enable facile purification and high solubility. Cleavage of the fusion tag using Factor Xa routinely resulted in heavy aggregation and associated low yields of the native kinase. 1 mg/ml k-MBP was prepared in cleavage buffer (20 mM Tris.HCl, 75 mM NaCl, 1 mM CaCl₂, pH 6.5) containing an increasing concentration of NV10, and then the protease Factor Xa was added to initiate cleavage of the MBP tag. The solutions were incubated at room temperature for 4 hours, and aggregation was monitored at intervals of 1 hour. The absorbance at 492 nm was used as a measurement of aggregation.

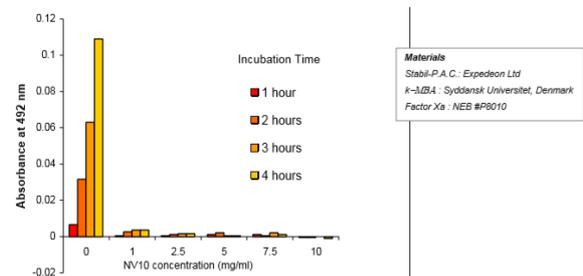


Chart 1 : Aggregation after tag cleavage of 1 mg/ml k-MBP

Cleavage of the fusion tag from k-MBP using a standard cleavage buffer results in severe aggregation and loss of protein yield as indicated by relatively high absorbance values at 492nm. The presence of NV10 in the cleavage buffer significantly reduces aggregation, and this is virtually eliminated at a ratio of 5 mg/ml NV10: 1 mg/ml protein or above

SUMMARY

NV10 can protect proteins from aggregation and loss of yield after fusion tag cleavage.

TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: technical.enquiries@expedion.com

For further information see our website: www.expedion.com