

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 Technology for Protein Mass Spectrometry

The generation of a sufficiently concentrated protein solution for applications such as mass spectrometry can be problematic due to protein aggregation and poor stability. NVoy Polymer, NV10, can be used to protect and stabilise proteins and is directly compatible with mass spectrometry techniques such as Electrospray Ionisation (ESI) and Matrix Assisted Laser Desorption Ionisation (MALDI). This contrasts with many other commonly used stabilisers (see Table 1) which are incompatible with either ESI or MALDI techniques and cannot readily be removed effectively.

**Refs:** Henze & Stults, Current Protocols in Protein Science (1996) 16.2.1-16.2.11. Ed. Coligan, J. E. Ogorzalek, *et al.* Meth. Mol. Biol. (1996) 61, 141-160. Ed. Chapman J.R.

Stabiliser	MALDI (wt.%)	ESI (wt.%)
Glycerol	1.2	n.a.
n-Hexyl-glucoside	n.a.	0.1
n-Octyl sucrose	n.a.	0.1
n-Dodecyl-glucoside	n.a.	0.1
PEG2000	0.1	n.a.
CHAPS	0.01	<0.1
Triton-X-100	0.1	<0.1
Zwittergent,3-16	0.1	n.a.
Tween20	0.1	<0.1
SDS	0.01	0.01
CTAB	n.a.	<0.1
<b>NV10</b>	<b>0.25</b>	<b>0.25</b>

**Table 1: Stabiliser Tolerance Chart**

### 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 Bradford*Ultra* assay, BCA assay, absorbance at 280nm).
2. ESI & MALDI typically require a 5  $\mu$ M solution of protein. This can be calculated using:

$$[\text{Protein}] \text{ (mg/ml)} = 5 \text{ (}\mu\text{M)} \times \text{formula weight (Da)} / 1000,000$$

3. Typically a fivefold excess, by mass, of NV10 will protect the target protein. For example, use 100  $\mu$ g/ml NV10 for 20  $\mu$ g/ml protein.
4. Each Stabil-P.A.C. tube contains 10mg NV10 as a lyophilised powder (40mg per tube in a Stabil-PAC MAXI).
5. 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 solution.
6. Concentrate the protein / NV10 solution to the desired protein concentration for mass spectrometry analysis.
7. 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.

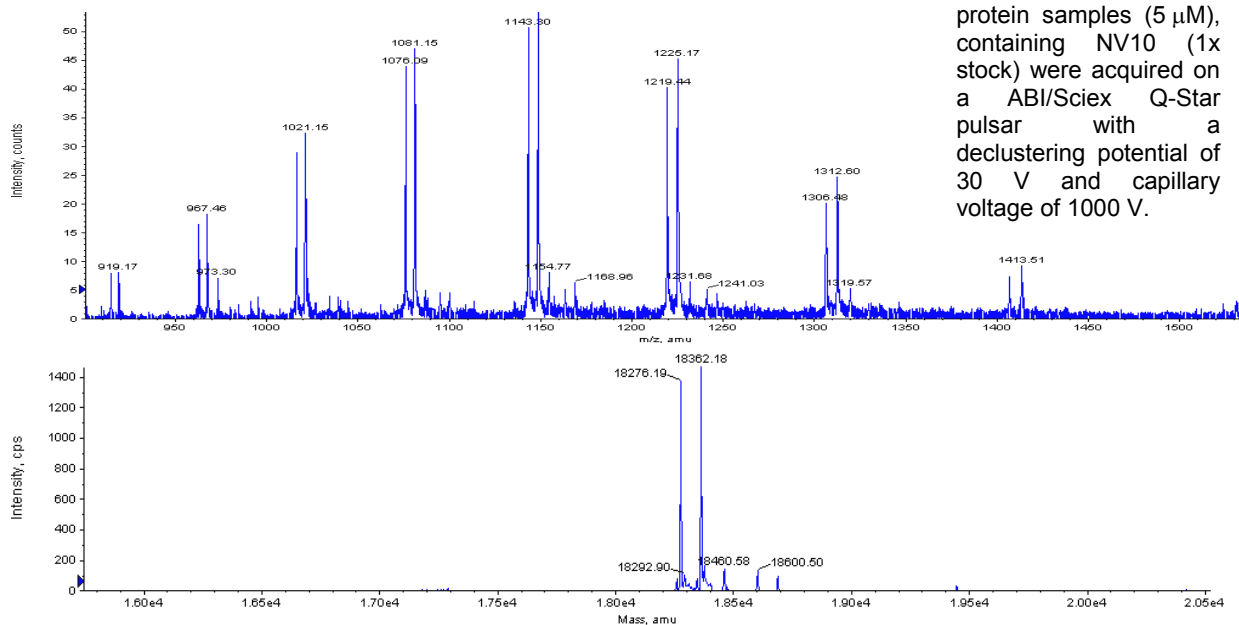
### Troubleshooting

Buffers which are required for protein purification frequently interfere with mass spectrometry techniques. In such cases the protein can still be purified, processed and concentrated in the presence of NV10 to prevent loss by aggregation and non-specific binding and then the contaminating buffers can be removed using Zip Tip (Millipore) purification immediately before sample application. (<http://www.millipore.com/publications.nsf/docs/tn072>)

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### Electrospray Ionisation (ESI)

ESI spectra of 0.1 mg/ml protein samples (5  $\mu$ M), containing NV10 (1x stock) were acquired on a ABI/Sciex Q-Star pulsar with a declustering potential of 30 V and capillary voltage of 1000 V.



The protein ionised well in the presence of NV10 (1x stock). Deconvolution of the spectrum gives two protein species corresponding to the masses of  $\beta$ -Lactoglobulin A and  $\beta$ -Lactoglobulin B

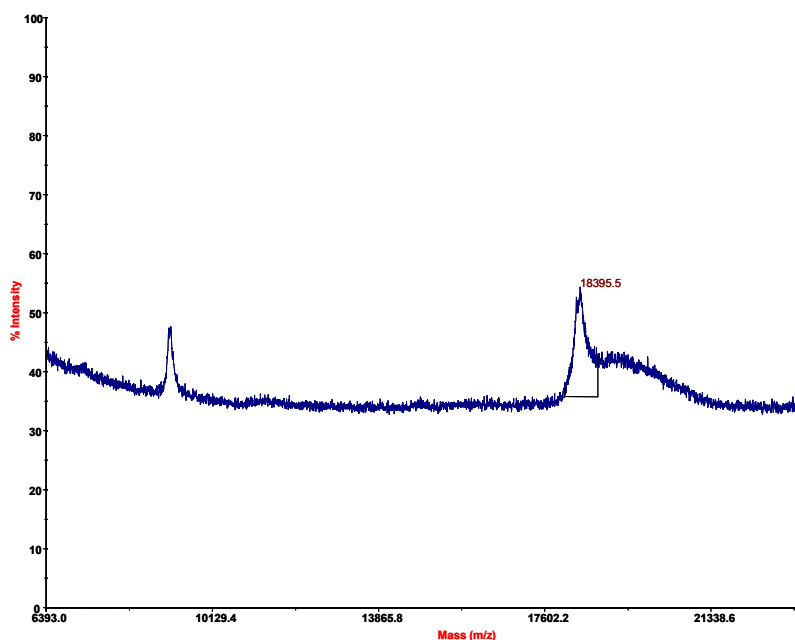
### Matrix Assisted Laser Desorption Ionisation (MALDI)

Maldi MS spectra of 0.1 mg/ml protein samples (5  $\mu$ M), containing NV10 (1x stock) were acquired on an ABI 4700 Proteomics Analyser with TOF/TOF optics. The mass range m/z 6000 - 23000 was analysed.

The protein sample was run in an  $\alpha$ -cyano-4-hydroxycinnamic acid matrix (10 mg/ml in 50% MeOH:water).

The aqueous protein sample was mixed in a 1 to 1 ratio with the matrix solution.

The protein ionised well in the presence of NV10 (1x stock) and a well defined protein peak was observed at 18.4 kDa, which corresponds to the mass of  $\beta$ -Lactoglobulin.



### Summary

Expedeon's NVoy technology enables the preparation of concentrated protein solutions suitable for direct application in mass spectrometers using either electrospray ionisation (ESI) or matrix assisted desorption ionisation (MALDI).