

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 in Protein Circular Dichroism (CD)

Biophysical analysis of protein solutions provides essential information about the structure and behaviour of a protein. Unfortunately protein instability can lead to denaturation or aggregation, which either prevents subsequent analysis or gives misleading data. The addition of NV10 to freshly prepared samples can stabilise protein solutions destined for circular dichroism spectroscopic analysis. NV10 has good transparency in the far-UV range and does not affect protein secondary structure, but can prolong protein stability and inhibit aggregation. Unlike detergents, NV10 is also compatible with analytical techniques including mass spectrometry and so samples prepared for CD are still suitable for subsequent analysis.

PROTOCOL

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

1. Determine the protein concentration (using eg. [BradfordUltra](#), [BCA assay](#), [absorbance at 280nm](#)).
2. Typically a fivefold excess, by mass, of NV10 will protect the target protein. For example, use 100 µg/ml NV10 for 20 µg/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 solution.
5. This protein / NV10 solution will be suitable for near and far-UV CD spectroscopy.
6. 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 the NV10 to protein concentration ratio can be increased, ie increase NV10 concentration and / or reduce protein concentration.
- Alternatively, a lower NV10 to protein ratio can be used with proteins which have no history of aggregation.
- Always measure buffer blanks with buffer containing NV10.

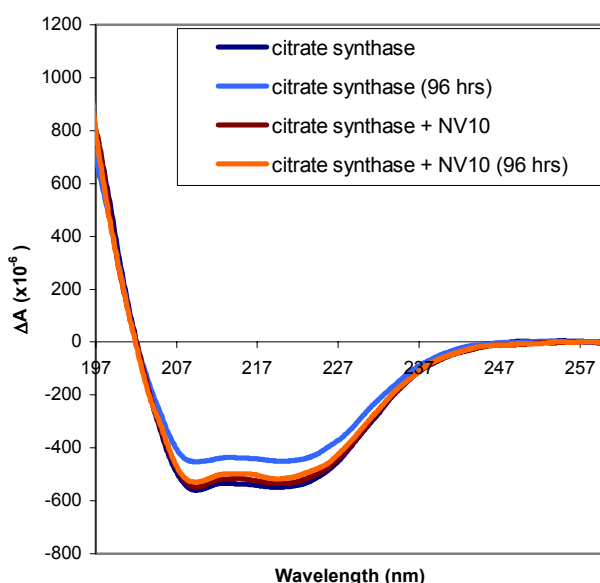
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EXAMPLE : Use of NV10 in CD Analysis

Citrate synthase catalyses the first step in the citric acid cycle. Samples stored in dilute solution at 4 °C gradually lose activity. This is accompanied by a decrease in intensity in the far-UV CD spectrum. The addition of NV10 to citrate synthase samples destined for CD analysis stabilises the protein without altering the secondary structure.

Citrate synthase was prepared at 18.6 µg/ml in either 5 mM Tris pH 7.8 alone, or in 5 mM Tris pH 7.8 containing 186 µg/ml NV10. Far-UV CD spectra of these samples were recorded at 22 °C in a 1 cm pathlength quartz cuvette. Buffer blanks containing either 5 mM Tris pH 7.8 alone, or 5 mM Tris pH 7.8 containing 186 µg/ml NV10 were also recorded, and the blank spectra were subtracted from the sample spectra. The samples were stored at 4 °C for 96 hours, then allowed to warm to room temperature and the far-UV CD spectra were measured again.

The initial far UV CD spectrum of citrate synthase was not affected by the presence of NV10, either by loss of transparency at low wavelength or perturbation of secondary structure. While the samples containing citrate synthase alone had lost signal intensity over the storage period suggesting loss of either material or secondary structure, the citrate synthase protected by NV10 retained virtually the full intensity of the far-UV CD signal. This observation is accompanied by reduction of the [enzyme activity for citrate synthase](#) alone, and retention of enzyme activity for citrate synthase in the presence of NV10 after storage for 96 hours at 4 °C.



Materials

Stabil-P.A.C. Expedeon Ltd
Citrate synthase : Sigma # C-3260
Jasco J-810 Spectropolarimeter
Jasco PFD-4256 Peltier

Figure 1: Far-UV CD spectra of citrate synthase stored in the presence or absence of NV10

Summary

NV10 can protect protein structure and maintain stability in solution prior to CD analysis.