



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, Improves Protein Recovery after Dialysis

Protein dialysis is a useful technique to enable a gentle change in protein buffer composition, the adjustment of salt and additive concentrations or the removal of low molecular weight impurities. Potential disadvantages of the technique include the loss of protein due to non-specific binding onto the dialysis membrane, and loss due to protein aggregation and instability during the intermediate stages of buffer exchange. The addition of NV10 to proteins before dialysis can minimise protein losses due to these factors and help to maximise protein recovery. NV10 is a large, linear polymer which dynamically associates with the target protein, masking any surface exposed patches of hydrophobicity, protecting it from aggregation and non-specific binding and improve protein stability. Although NV10 is in dynamic equilibrium with the protein its large size (Hydrodynamic radius around 18 kDa) means that it is typically retained within the dialysis membrane and is therefore not required in the dialysis buffer.

PROTOCOL

Aggregation, non-specific binding 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 280 nm).
2. Typically a fivefold excess, by mass, of NV10 will protect the target protein. For example, use 100 µg/ml NVoy 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 NVoy 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. Dialyse this protein / NVoy solution.
6. NVoy associates with the protein in solution and is retained by the membrane during dialysis to give continuing protection further downstream.
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

- If the protein shows signs of aggregation or heavy losses 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.
- Protein solutions dialysed using membranes with pore sizes of greater than 10,000 kDa may gradually lose NV10 into the dialysis buffer.



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EXAMPLE : Use of NV10 in Dialysis

A stock solution of 1 mg/ml β -lactoglobulin in 50 mM Tris, 0.15 M NaCl pH 8.0 (TS buffer) was prepared, along with a 2.5 mg/ml solution of NV10 in TS Buffer. Samples were prepared in duplicate containing 10 μ g/ml of β -lactoglobulin either in TS buffer alone, or in TS buffer containing either 10 μ g/ml or 100 μ g/ml NV10. 2 ml of each sample was loaded into a Pierce Slide-A-Lyser dialysis cassette (10,000 mwco) according to the manufacturer's protocol, and dialysed overnight at 20 °C against TS buffer. The volume of the protein recovered was measured and the protein concentration was estimated using Expedeon's BradfordUltra reagent.

Initial protein concentration	Recovered protein (% yield)
10 μ g/ml β -lactoglobulin	77%
10 μ g/ml β -lactoglobulin + 10 μ g/ml NV10	85%
10 μ g/ml β -lactoglobulin + 100 μ g/ml NV10	100%

Table 1 : Recovery of β -lactoglobulin after dialysis

The protein losses associated with dialysis decrease in the presence of increasing NV10 concentrations. Full recovery of β -lactoglobulin was achieved after dialysis in the presence of 100 μ g/ml NV10.

Materials

Stabil-P.A.C.: Expedeon Ltd

BradfordUltra: Expedeon Ltd

β -Lactoglobulin : Sigma L-0130

Slide-A-Lyser Dialysis Cassettes, 10,000 mwco: Pierce PerbioScience

Summary

NV10 can protect proteins from aggregation and loss to membranes during dialysis.