

NVoy Tech Note

Protein Concentration

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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.

NV10 IMPROVES RECOVERY AFTER PROTEIN CONCENTRATION

Protein aggregation is strongly dependent on protein concentration, and so the preparation of a concentrated protein solution can be problematic with associated protein aggregation and membrane losses. The build-up of protein aggregates on the surface of the membrane filter during filtration and the membrane polarization layer can increase resistance to filtrate flow and decrease filtration efficiency. Expedeon's NV10 polymer protects the target protein and stabilises the protein at increasing concentrations helping to keep it monomeric and soluble, as well as minimising protein losses to hydrophobic surfaces and speeding up filtration.

PROTOCOL

Aggregation, stability and non-specific binding are 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 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. Concentrate the protein / NV10 solution to the desired protein concentration.
6. NV10 will co-concentrate with the protein in solution to give continuing protection as the protein concentration increases.
7. NV10 stock solutions (up to 10 mg/ml) can be stored for up to 1 week at 40C 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.

EXAMPLE:

Use of NV10 in Protein Concentration:

A stock solution of 1 mg/ml BSA in PBS was prepared, along with a 2.5 mg/ml solution of NV10 in PBS. These stocks were used to prepare duplicate samples containing 10 µg/ml BSA in PBS and supplemented with increasing concentrations of NV10. 1 ml aliquots of each sample were placed into Vivaspin 2 spin concentrators (5,000 mwco, Hydrosart low protein-binding membrane) and concentrated tenfold according to the manufacturer's instructions. The concentrate was collected using reverse spin, and the recovered protein content was measured using Expedeon's BradfordUltra reagent.

STARTING SOLUTION (1 ML)	RECOVERED YIELD (%)
10 µg/ml BSA	46 %
10 µg/ml BSA + 10 µg/ml NV10	60 %
10 µg/ml BSA + 40 µg/ml NV10	85 %
10 µg/ml BSA + 100 µg/ml NV10	90 %

Table 1: Recovered protein yields following tenfold spin concentration.

Increasing NV10 concentration during spin concentration of BSA has a marked effect on recovery, with 100 µg/ml NV10 doubling the recovered yield of BSA.

Materials Stabil-P.A.C.: Expedeon Ltd
 BradfordUltra: Expedeon Ltd
 BSA : Fluka #05477
 Vivaspin2, 5000 mwco HY: Sartorius

SUMMARY

NV10 can increase protein recovery after spin concentration.

TECHNICAL SUPPORT

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

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