



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 Improves Protein Recovery on PD10 Desalting Columns

Protein desalting is generally a high-yielding procedure, however protein losses do regularly occur when working with sticky, aggregation-prone or dilute protein solutions. The addition of NV10 to such solutions can considerably reduce losses onto column media resulting in improved yields.

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 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. Continue with PD10 desalting of protein + NV10 solution as normal.
6. NV10 will co-elute with the protein in solution to give continuing protection 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 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 that have no history of aggregation.



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EXAMPLE : Use of NV10 With PD10 Columns

A stock solution of 1 mg/ml BSA in 50 mM Tris, 0.15 M NaCl pH 8.0 (TS buffer) was prepared, along with a 1X solution of NV10 (2.5 mg/ml). Samples were prepared in duplicate containing either 10 µg/ml of BSA in TS buffer alone or 10 µg/ml of BSA in TS buffer containing 100 µg/ml NV10. 2.5 ml of each sample was loaded onto a PD10 column according to the manufacturer's protocol, and eluted in 3.5 ml of TS buffer. The total protein recovered was measured using Expedeon's BradfordUltra solution.

PD10 sample	% BSA recovery in PD10 eluate
10 µg/ml BSA	85 %
10 µg/ml BSA + 100 µg/ml NV10	96 %

Table 1: BSA recovery after PD10 desalt column

Proteins are often lost due to non-specific binding, especially at low working concentrations, and even a "model" protein such as BSA can experience up to 15 % loss of protein on a PD10 desalting column. Addition of 100 µg/ml NV10 minimises non-specific binding, and enables virtually full recovery.

Materials

Stabil-P.A.C.: Expedeon Ltd

BradfordUltra: Expedeon Ltd

BSA : Fluka #05477

PD-10 columns: GE Healthcare

Summary

NV10 can improve protein recovery from PD10 desalting columns.