

# NVoy Tech Note - PD10 protocol

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## INTRODUCTION

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.

## 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.

## SUMMARY

NV10 can improve protein recovery from PD10 desalting columns.

## TECHNICAL SUPPORT

For technical enquiries get in touch with our technical support team at: [technical.enquiries@expedeon.com](mailto:technical.enquiries@expedeon.com)

For further information see our website: [www.expedeon.com](http://www.expedeon.com)