

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.

Removal of NVoy Polymer, NV10, From Samples

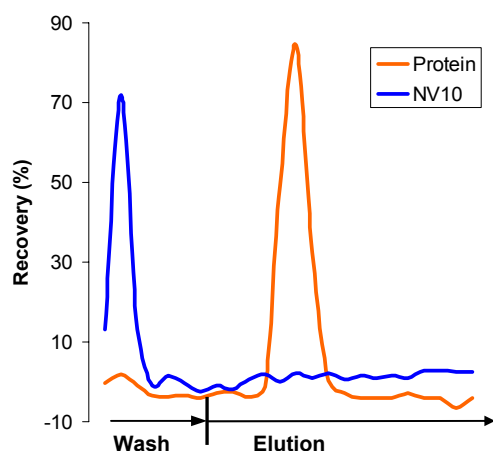
NVoy polymer, NV10, interacts with hydrophobic patches on a protein forming a polymer : protein complex in dynamic equilibrium with the free polymer in solution (e.g. $k_d = 28 \mu\text{M}$ with GFP). NV10 can be removed from a protein solution by binding the target protein to ion-exchange or affinity media and washing out NV10 before elution of the target protein.

Ion exchange chromatography (IEx) is a technique whereby a charged protein can reversibly interact with an oppositely charged matrix, and then be eluted by a change in buffer ionic strength or pH. Each protein is composed of a unique combination of individual amino acids, and different proteins therefore often have differing net charges at any given pH.

- The pH at which a protein has no net charge is termed the iso-electric point (pI).
- The pI of a protein should be determined experimentally, but if the amino acid sequence is known then the theoretical pI can be estimated at websites such as <http://us.expasy.org/tools/>.
- Under conditions where the pH is higher than the pI the protein will have a net negative charge, and bind to an anion exchange resin (positively charged).
- Under conditions where the pH is lower than the pI the protein will have a net positive charge and bind to a cation exchange resin (negatively charged).

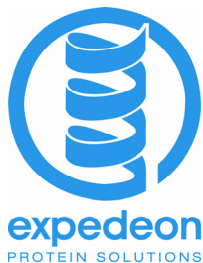
Affinity chromatography is a method of separating a mixture of proteins by utilizing specific interactions of the target protein with a ligand which is immobilized on a support, eg IMAC, Protein A, Glutathione.

PROTOCOL FOR REMOVAL OF NV10



1. Bind the target protein to ion exchange or affinity chromatography media under conditions appropriate to the target protein.
2. Wash the medium with a minimum of 10 column volumes of binding or wash buffer to remove NV10.
3. Elute the target protein using increasing ionic strength, pH gradient or a competing ligand (affinity chromatography).

Figure 1: Typical profile illustrating separation of NV10 from target protein using IEx or affinity chromatography.



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Troubleshooting

In most cases the target protein will bind as normal to the resin of choice. If the binding is weaker than expected the interaction with the solid phase can be enhanced using a release agent to weaken the interaction between the protein and NV10 polymer. Expedeon supplies these release agents as part of Stabil-P.A.C. and NVoy Polymer Pack Kits:

- Addition of dimethylsulfoxide (DMSO) will facilitate a slow gentle release.
- Addition of "NV10 Strong Release Agent" will facilitate instantaneous release.

PROTOCOL FOR RELEASE OF NV10

1. Determine quantity of NV10 in solution.
2. Up to 10 % of release agent (either DMSO, or 1X Strong Release Agent) may be added to a 1X solution of NV10 (2.5 mg/ml).
3. Incubate for 10 minutes room temperature.
4. Perform NV10 removal using IEx or affinity chromatography.

Note! A protein which is intrinsically prone to aggregation or instability may precipitate at this point as the protection afforded to it by NV10 will be removed.

Troubleshooting

If a heavy protein precipitate forms at the release step:

- Use DMSO as a release agent rather than "NV10 Strong Release Agent" to give a more gentle dissociation of components.
- Add less release agent.
- Add release agent in small aliquots over a longer period of time.
- Perform release at 4 °C.

If NV10 is still present in the protein sample after release and removal:

- Extend column chromatography wash step to 20 column volumes before protein elution.
- Add 10 % DMSO to the IEx or affinity wash buffer before protein elution.

E-mail scientist@expedeon.com or call +44 (0)1223 496500 for
Immediate, expert and confidential technical support.