

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.

The Application of NVoy Technology to Protein Stabilisation

A stable and reproducible protein standard curve is essential for applications such as enzyme production and ELISA assays. Unfortunately some proteins have poor stability within the working range of the standard activity assay. The addition of NVoy Polymer, NV10, to enzyme or protein standard stocks can stabilise standards and contribute towards long term reliability, without compromising activity or protein structure.

PROTOCOL

Stability is very protein specific, but a general protocol is given below.

1. Determine the protein concentration (using eg. Expedeons Bradford*Ultra* Assay, BCA assay, absorbance at 280nm).
2. Typically a fivefold excess, by mass, of NV10 will protect the target protein. For example, use 200 µg/ml NV10 for 40 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. This protein / NV10 stock solution can now be used in subsequent dilutions to prepare sets of protein standards.
6. 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 which have no history of aggregation.
- Always measure assay blanks with buffer containing NV10.

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EXAMPLE : Use of NV10 to Improve Enzyme Stability

The activity assay for citrate synthase is very sensitive, detecting protein in the $\mu\text{g/ml}$ range. Standard activity curves prepared using commercial citrate synthase require the stock protein to be highly diluted in 0.1 M Tris pH 7.8 in order to fall within the range of the assay. Unfortunately the resulting standard series is unstable, and activity is quickly lost at the lower end of the assay. This requires the preparation of fresh standards for each experiment or time point. The addition of NV10 to the citrate synthase stock before dilution results in a set of standards that is stable for a prolonged time at 4 °C, saving time, maintaining reproducibility and reducing protein costs.

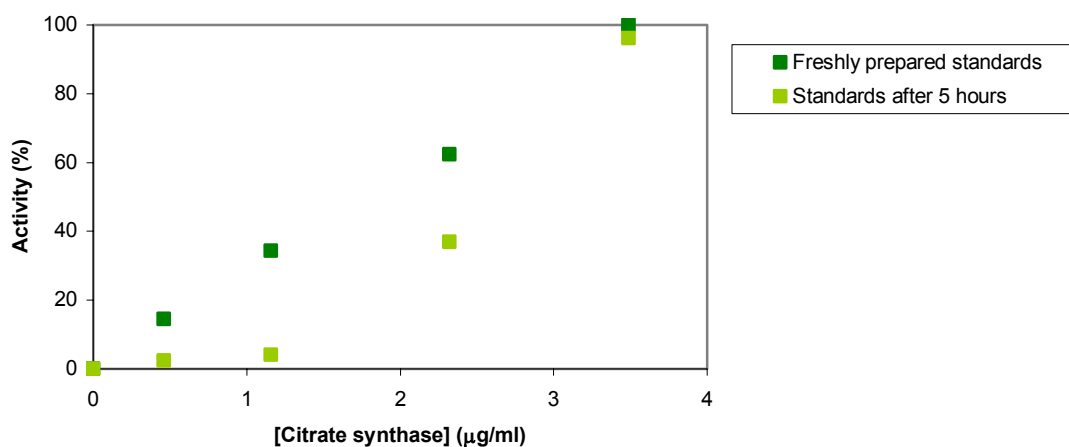


Figure 1: Within 5 hours of preparing the citrate synthase set of standards the standard curve has lost linearity, and the activities of the standards at 0.4 $\mu\text{g/ml}$ and 1.1 $\mu\text{g/ml}$ have virtually disappeared.

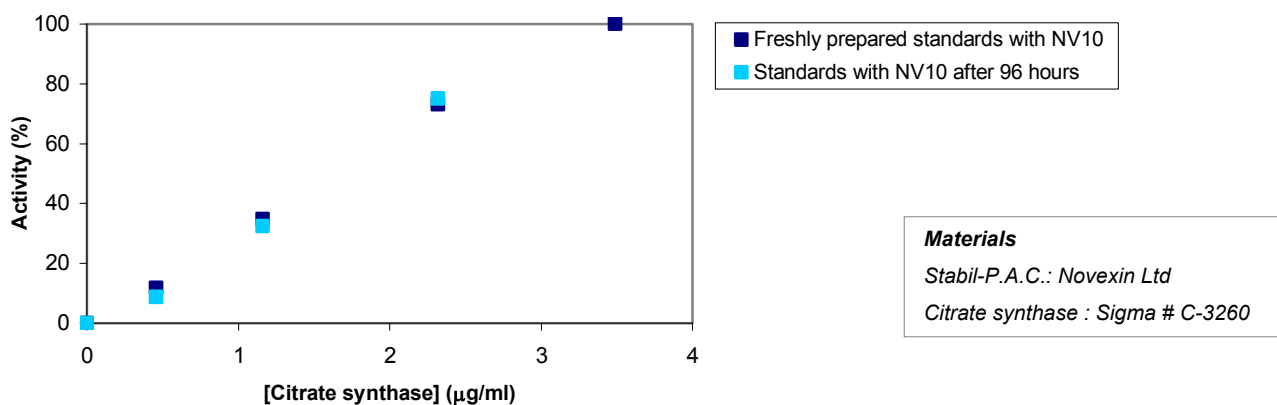


Figure 2: By contrast, the samples of citrate synthase prepared with NV10 are still fully active, producing a linear standard activity response even after 96 hours.

Summary

The addition of NV10 to unstable protein standards can result in a standard set which gives reliable and reproducible results over several days.