Stabil-P.A.C v1.2

Introduction

Protein processing and production is often hampered by the formation of aggregates that restrict and complicate the development of proteins, antibodies and enzymes. Stabil-PAC is designed to minimise the sequential losses in consecutive protein processing steps which would otherwise dramatically reduce the overall protein yield.

Utilising NVoy technology, Stabil-PAC is an alternative to the use of detergents, fusion proteins, arginine, chaperones and a range of other common additives employed to increase protein solubility and enable the handling of proteins in solution.

Each kit contains proprietary carbohydrate polymers, NV molecules, designed to increase the solubility and stability of proteins whilst preventing aggregation and reducing non-specific binding. NV molecules are linear carbohydrate polymers, derivitised to make them highly amphipathic. They are uncharged molecules of around 5kD. They associate at multiple points with the hydrophobic patches of proteins in a dynamic fashion to form multipoint reversible complexes. Multiple-binding points allows NV molecules to be used at low concentrations relative to alternative reagents and their size prevents them from entering the protein core and inhibiting normal structural bonding or blocking catalytic/binding sites. Being based on simple carbohydrate polymers they are easily separated from the protein when they are no longer required in solution.

The impact of NVoy technology can be seen in many areas of protein research including stabilisation, purification, analysis and crystallisation.

For case studies on NVoy applicability please visit our website at www.novexin.com

Contents

1) NV10 (8x solid, blue cap)

- Supplied lyophilised in 8 aliquots
- sufficient to protect 8x 0.5 ml of protein solution (Target protein concentration 1 mg/ml)

2) Dimethylsulfoxide (DMSO, 0.5 ml, 1x, red cap)

3) NV10 removal solution (0.5 ml, 1x, green cap)

- Supplied in ultrapure water.

Storage

Upon receipt store at + 4°C. Discard any reagents that show discoloration or evidence of microbial contamination.

NV10 is stable as supplied but should be used within one week of initial solution preparation. Reconstituted NV10 can be temporarily stored at -24 °C.

Recommended Protocol and Notes on NV10 Usage

1) To obtain a 1x NV10 solution, resuspend the lyophilised NV10 in 500 µl of your desired aqueous buffer / protein solution (target 1mg/ml protein).

2) If protein aggregation occurs increase the NV10 concentration or decrease the final protein concentration.

Notes on use of Release Agents

Two release agents have been provided to allow you to control the interaction between the NV10 molecules and your protein.

- Dimethylsulfoxide (DMSO) is a weak release agent and will facilitate a slow, gentle release.

- The NV10 removal solution is a strong release agent and will facilitate rapid release.

For a solution containing 1x NV10, up to 10% of the chosen release agent may be added.

Frequently Asked Questions

Q 1 Can I make a more concentrated solution of NV10?

A 1 NV10 can be dissolved up to a 20x solution in any common aqueous buffer system. However, the NV10 crystallisation will occur faster in more concentrated solution (See Q 3).

Q 2 Is the NV10 solubility pH dependent?

A 2 The NV10 solubility is not pH dependent. NV10 can be dissolved in aqueous buffers with pH ranging from 3 to 11.

Q 3 Can I store a solution of resuspended NV10.

A 3a When NV10 is resuspended in an aqueous buffer the NV10 molecules will slowly crystallise. This will, in time, lead to a cloudy solution. As a rule of thumb a pure 1x NV10 solution is stabile for at least one week at 4 °C. More concentrated solutions will appear cloudy more rapidly. The NV10 crystallisation process only occurs for pure NV10 solutions. Moreover, despite the undesirable cloudy appearance, a crystallized NV10 solution will retain its functionality.

A 3b When NV10 is resuspended in an aqueous buffer it is ideally used immediately to protect your proteins. However, solutions of resuspended NV10 can also be temporarily stored at -24 °C.

Q 4 Are any purification steps necessary before protein analysis?

A 4 A 1x solution of NV10 is compatible with many analytical techniques.

Protein Concentration	
BCA Assay	✓
Bradford Assay*	✓
UV spectroscopy	✓
Protein Structure	
Circular Dichroism	✓
Mass spectrometry**	✓
Column Chromatography	
IMAC	✓
Ion Exchange	✓
Reversed Phase***	✓
Protein Activity	
Cell Based Assays	✓
ELISA Assays	✓
FRET assays	✓

* Use a blank containing NV10. NV10 will give a weak Bradford signal. The assay will show a reduced sensitivity for protein concentrations lower than 0.25 mg/ml.

** Standard C4 zip tip clean up recommended

*** Use of guard column recommended

However, it may be desirable to carry out protein purification steps before gel-electrophoresis, Bradford analysis and ELISA since more accurate results may be obtained.

Q 5 Which is the preferred method to remove NV10 from my protein solution?

A 5 The recommended purification method is ion-exchange chromatography, or immobilised metal affinity chromatography (IMAC) if the protein has a histidine tag. Hydrophobic interaction chromatography can be used as a secondary protein polishing procedure if complete removal of the NV10 is required.

Q 6 My protein only binds the chromatography resin weakly. Can I improve the binding?

A 6a To enhance the binding to the chromatography resin, release agents may be added to the sample. However, adding release agents will reduce the protein protection and may lead to higher protein losses on the column.

A 6b The actual protein properties may differ from theoretical protein properties. Try screening different ion exchange resins and buffers at different pH to find more suitable binding conditions.

Q 7 Can I use membranes, size exclusion chromatography or desalt resins to separate my protein from NV10 molecules?

A 7a No, these purification strategies are not recommended since NV10 has a large hydrodynamic radius and may therefore co-elute with your protein in size-based separations.

A7b To enable size-based purification strategies release agents can be used to weaken the NV10 protein protection. However, by doing

so the protein will no longer be protected, which may result in protein losses.

Q 8 I have uncapped both release agents and don’t remember which one is which. How can I find out?

A8 Close the tubes and put both tubes in the fridge for 1h. The tube for which the content solidifies is DMSO.