Protein Purification Overview

Protein Extraction

When purifying proteins from cell or tissue sources, the first step is disruption of the sample and extraction of the relevant protein fraction. This step is often critical because processing methods that require harsh mechanical and/or enzymatic treatments can directly affect the target protein's integrity and/or activity, or otherwise expose it to degradative conditions. To address this problem, Novagen has introduced BugBuster™ and CytoBuster™ Protein Extraction Reagents, which are novel combinations of detergents and other ingredients that enable gentle, efficient, non-mechanical extraction of soluble proteins from bacteria and mammalian cells, respectively. The addition of Benzonase® Nuclease specifically degrades contaminating DNA and RNA for the preparation of non-viscous, nucleic acid-free extracts ready for target protein purification. Protease Inhibitor Cocktails are available for protection of the target protein against degradation in crude extracts.

Affinity Purification

Affinity purification is based on the specific interaction of a target molecule with an immobilized ligand. Affinity technology may be used to isolate specific molecules from a mixture (e.g., His•Bind® purification), capture a desired molecule for interaction studies (e.g., immobilization on CBIND™ Resin), or remove a component from a reaction (e.g., protease removal in the Cleavage Capture Kits). For recombinant proteins, the addition of fusion tags using appropriate expression vectors enables affinity purification by a number of strategies. Novagen offers the widest selection of immobilized metal affinity chromatography (IMAC) matrices for purification of His•Tag® fusion proteins, including agarose-based His•Bind Resin, pre-charged, non-leaching Ni-NTA His•Bind Resin, His•Bind Magnetic Agarose Beads, cellulose-based pre-charged His•Bind Quick Columns and Cartridges, and methacrylate His•Bind Fractogel®, which enables higher pressures and capacities. Other agarose-based affinity resins enable rapid purification of T7•Tag®, S•Tag™ and GST•Tag™ fusion proteins, and a variety of CBIND cellulose formats are available for purification of CBD•Tag™ fusion proteins. Restriction grade site-specific proteases (Thrombin, Biotinylated Thrombin, Factor Xa and Recombinant Enterokinase) and Cleavage Capture Kits for each enzyme offer convenient removal of fusion tags. EKapture™ and Xarrest™ Agaroses are used for the quantitative removal of rEK and Factor Xa, respectively, following cleavage of fusion proteins.

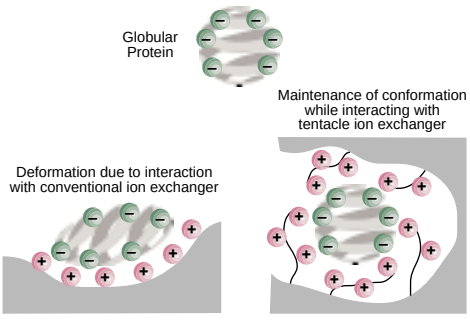
For the convenient, reproducible preparation of custom affinity supports containing antibodies, peptides, proteins or other user-defined ligands, Novagen offers three activated matrices that are ready for coupling. PreACT™ Agarose ALD is a preparation of aldehyde-activated cross-linked agarose that forms stable amide linkages with free amino groups of proteins or peptides in the presence of sodium cyanoborohydride. Two other PreACT resins are based on the Fractogel® EMD tentacle backbone, which provides extremely high capacities, low non-specific binding and superior mechanical properties. PreACT Fractogel AZL enables coupling of proteins in one step under very gentle conditions, and PreACT Fractogel EPX is designed for high-density coupling of alkaline-stable compounds.

Streptavidin Agarose and MagPrep® Streptavidin Beads provide a choice of formats for purification of biotin-containing molecules. Both supports are qualified for high biotin binding activity and low non-specific background. Protein A and Protein G agaroses are indispensible tools for affinity purification and immunoprecipitation of antibodies and their bound antigens. Two types of supports are available that contain highly purified Protein A and/or Protein G Plus; one is designed for purification and the other for immunoprecipitation. Protein A is also available in the form of PANSORBIN® fixed *Staphylococcus aureus* cells, which is an extremely versatile and widely used reagent for immunoprecipitation.

In addition, Novagen is pleased to offer a complete range of anti-immunoglobulin magnetic beads. The MagPrep Anti-Ig Beads consist of a superparamagnetic iron oxide core encapsulated within a polystyrene shell coated with antibodies against mouse, rat, or human IgG or IgM. These beads offer many advantages for highly selective capture and immobilization of antibodies and immune complexes.

Fractogel EMD Tentacle Resins

The Fractogel EMD Tentacle Resins provide a wide array of chemistries for conventional biochromatography as well as affinity applications. The Fractogel matrix is a widely used methacrylate resin upon which polyelectrolyte chains (tentacles) have been grafted. The tentacles provide a flexible, hydrophilic backbone for the attachment of ion exchange groups, affinity ligands, hydrophobic moieties, or other functional groups useful for chromatography. In contrast to conventional chromatographic beads, the flexible tentacles on Fractogel allow great increases in resin capacity. In addition, the tentacles can conform to the bound analyte molecule, allowing for tighter interactions and improved resolution. Because the interactions between analyte and tentacle are not limited to the bead surface, great improvements in performance including reduced non-specific binding are typically observed when Fractogel resins are compared to conventional beaded chromatography resins.



The Fractogel bead organic polymeric structure has very good stability under extremes of pH and high pressure (> 250 psi). It is also suitable for use with some organic solvents. Fractogel particles are resistant to enzymatic degradation. In addition, for large bioprocessing applications Fractogel resins are manufactured under GMP guidelines and fully compatible with clean in place (CIP) procedures.

Purification Overview

Solid Phase Supports for Protein Purification

Product Category	Composition	Particle Size (µm)	Max. Pressure	Products	Basis of Separation	Page
Agarose-based affinity resins	Cross-linked beaded agarose	45–165	2.8 psi	His•Bind® Resin	His•Tag® affinity (IMAC)	154
				Ni-NTA His•Bind Resin	His•Tag affinity (IMAC)	154
				T7•Tag® Antibody Agarose	T7•Tag affinity	159
				S-protein Agarose	S•Tag™ affinity	160
				GST•Bind™ Resin	GST•Tag™ affinity	158
				Streptavidin Agarose	Biotin affinity	165
				EKapture™ Agarose	Enterokinase affinity	163
				Xarrest™ Agarose	Factor Xa affinity	164
				Protein G Plus Agarose	IgG affinity	166
				Protein A Agarose	IgG affinity	166
				Protein G Plus/Protein A Agarose	IgG affinity	166
				PreACT™ Agarose ALD	User-provided ligand affinity	161
		60–160	140 psi	Ni-NTA His•Bind Superflow	His•Tag affinity (IMAC)	154
Fractogel® EMD Tentacle resins	Methacrylate polymer	20–90	267 psi	TMAE, TMAE HiCap, DEAE, DMAE	Anion exchange	169
				SO_3, SE HiCap, COOH	Cation exchange	169
				Propyl, Phenyl	Hydrophobic interaction	170
				TA	Thiophilic adsorption	170
				BioSEC	Size exclusion	169
				His•Bind	His•Tag affinity (IMAC)	154
				PreACT™ AZL, PreACT EPX	User-provided ligand affinity	161
Cellulose-based resins	Beaded cellulose	100–200	50 psi	His•Bind Quick cartridges/columns	His·Tag affinity (IMAC)	154
				CBIND™ resins/cartridges/columns	CBD•Tag™ affinity	156
Hydroxylapatite	Crystalline calcium phosphate	10–50		Hydroxylapatite, Fast Flow	Ion exchange plus adsorption	171
		50–200		Hydroxylapatite, High Resolution		
Magnetic beads	Polystyrene coated ferric oxide	Polydisperse; average 1 µm		His•Bind Magnetic Agarose Beads (3 µ)	His•Tag affinity (IMAC)	154
				MagPrep® Streptavidin Beads	Biotin affinity	165
				MagPrep Anti-Mouse IgG Beads	IgG affinity	167
				MagPrep Anti-Mouse IgM Beads	IgM affinity	167
				MagPrep Anti-Rabbit IgG Beads	IgG affinity	167
				MagPrep Anti-Rabbit IgM Beads	IgM affinity	167
				MagPrep Anti-Human IgG Beads	IgG affinity	167
				MagPrep Anti-Human IgM Beads	IgM affinity	167
PANSORBIN®	Fixed *S. aureus* cells	1–2	batch only	PANSORBIN Cells	IgG affinity for immunoprecipitation	165

Protein Refolding

Some purification strategies take advantage of the formation of insoluble inclusion bodies, which can sequester the target protein and protect it from degradation in the cell. To obtain the target protein in an active, soluble form, the inclusion bodies must be solubilized and the protein refolded. While the optimal conditions for solubilization and refolding largely depend on the individual protein, there are some general protocols and reagents that have been successfully used with a number of different proteins. The Protein Refolding Kit combines the reagents to perform one such protocol and can serve as a platform for using additional reagents, such as the NDSBs, for optimization.