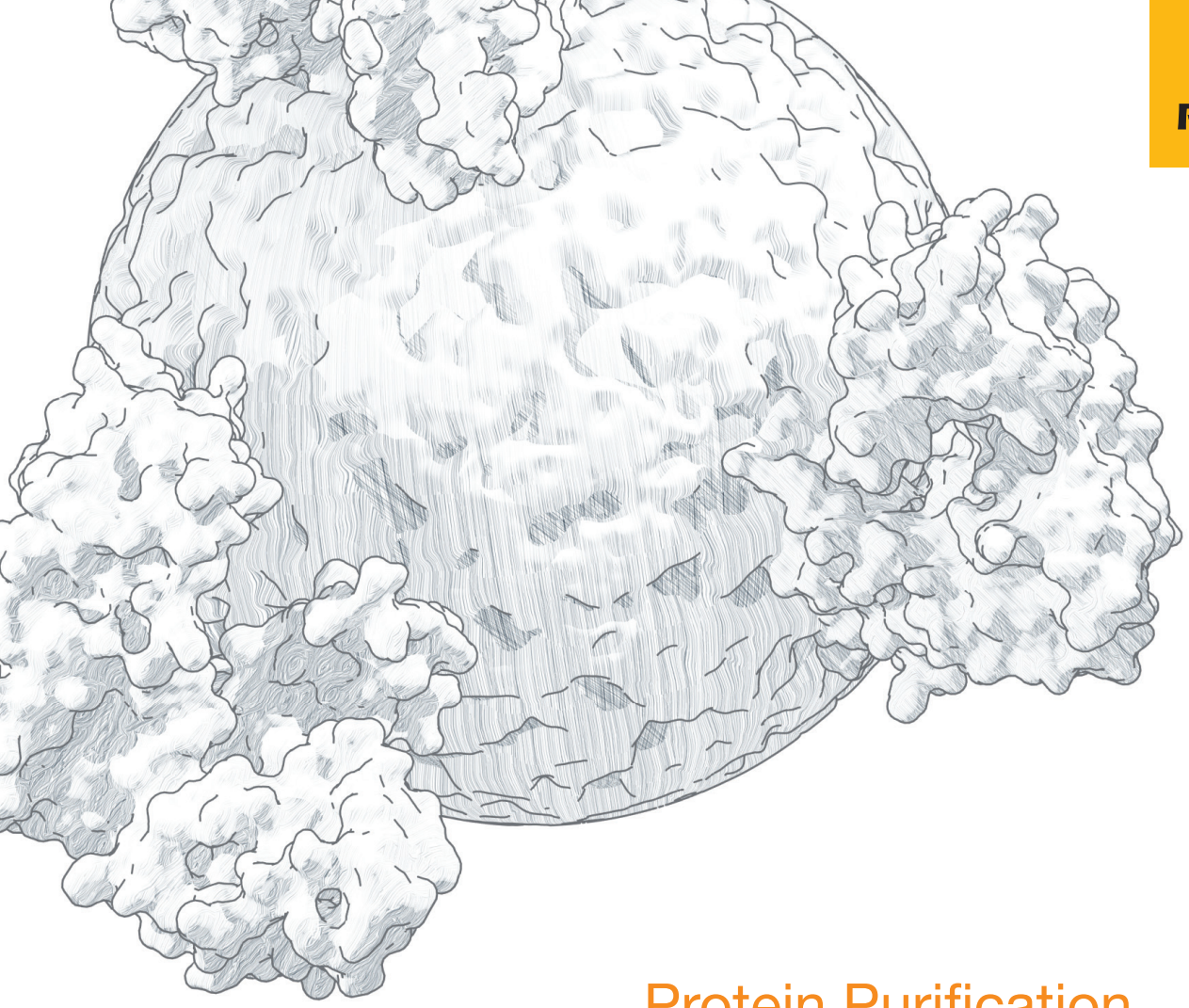
4 Protein Purification



Protein Purification

Protein purification is fundamental to the study of protein function and involves a series of processes to express, enrich and purify a protein of interest from a complex mixture such as cell lysate. The fastest and most powerful method for this purpose is affinity purification. During the purification of recombinant proteins it is convenient to use affinity tags such as HaloTag, GST- or His-tag. By introducing a cleavage-site between the protein fusion tag and the protein sequence, the tag can be enzymatically removed after the purification procedure.

In this chapter different affinity tags will be introduced as well as the corresponding resins and magnetic supports that can be used for low- to high-throughput protein purification.

4.1 Affinity-based Protein Purification

OVERVIEW

Affinity purification is a powerful method to enrich and purify a recombinant protein from a cell lysate. For this purpose an affinity tag is placed either on the N- or C-terminus of the protein of interest. There are numerous affinity tags that can be used for protein purification; the most common tags are listed in **Table 4.1**. Affinity tags vary greatly based on size, strength-of-binding and effect on protein solubility and stability.

The first step to purification of a recombinant protein is the preparation of the cell lysate or supernatant. Cell lysis can be accomplished using a variety of methods, including nonenzymatic methods (e.g., sonication or French press) or the use of hydrolytic enzymes, such as lysozyme (for *E. coli*) or detergent reagents such as FastBreak™ Cell Lysis Reagent. For secreted proteins, minimal supernatant preparation is required, followed by selective binding, washing and elution of the purified protein. After purification the protein may be cleaved with a protease to remove the affinity tag.

Table 4.1. Comparison of Properties of Commonly Used Affinity Tags for Protein Purification.

Tag	Size (kDa)	Affinity Strength	Solubility Enhancement
His	~1	++	–
Strep	~1	+++	–
FLAG HA	~1	+++	–
GST	~26	+	++
MBP	~42	+	+++
HaloTag	~34	++++	+++
SUMO	~12	+	+++

Types of Affinity Tags

Polyhistidine (His) Tags

The most commonly used tag to purify and detect recombinant expressed proteins is the polyhistidine tag. Protein purification using His tags relies on the affinity of histidine residues for immobilized chelated metal such as nickel, which allows selective protein purification. The metal is immobilized and is covalently attached to a solid support such as agarose beads.

His tags offer several advantages for protein purification. The small size of the His tag makes it less immunogenic than larger tags, although it does not enhance the solubility of the fused proteins. The His tag can be placed on either the N- or C-terminus of the protein of interest. And finally, the interaction of the polyhistidine tag with the metal support material does not depend on the tertiary structure of the His tag, making it possible to purify proteins under denaturing conditions such as 8M urea and 6M guanidine hydrochloride. Post binding and washing, the protein is eluted with a high concentration of imidazole, (greater than 100mM) or with other elution methods, including low pH, and EDTA.

Biotin Tags

The power of the streptavidin-biotin interaction is used in a number of applications, including the detection of various biomolecules. Bacterial streptavidin and its analog chicken avidin have a very high binding affinity for biotin (vitamin H). In fact, this is one of the strongest non-covalent interactions known in biology. The strength of the binding is a major drawback for its use as a purification technique, since the elution conditions would have to be so harsh that they would destroy the purified protein.

Nevertheless, there are several ways to circumvent this strong binding and harness the power of the streptavidin-biotin interaction for the purification of recombinant proteins. One strategy is to fuse the protein of interest (POI) to a peptide sequence that is biotinylated in vivo. The biotinylation peptide sequence is fused to a protease cleavage site allowing a gentle release of the protein of interest by protease digestion. Other strategies are based on biotin-mimicking peptides such as Strep Tag, which binds to native and modified versions of streptavidin and avidin, enabling successful protein elution under mild conditions (e.g., competitive elution with D-desthiobiotin).

Glutathione-S-Transferase (GST)

The use of the affinity tag glutathione-S-transferase (GST) is based on the strong affinity of GST for immobilized glutathione-covered matrices. Glutathione-S-transferases are a family of multifunctional cytosolic proteins that are present endogenously in eukaryotic organisms but normally not found in bacteria. As such, GST-based purification is not recommended for eukaryotic systems (e.g., insect, mammalian). The 26kDa GST affinity tag enhances the solubility of many eukaryotic proteins expressed in bacteria. After capture and washing, the tagged protein is eluted with soluble glutathione.

HaloTag® Protein Tag

Protein fusion tags are frequently used to aid in expression of suitable levels of soluble protein as well as for purification. The HaloTag® fusion protein is engineered to enhance expression and solubility of recombinant proteins in *E. coli*, as well as to provide superb recovery of even low-expressing proteins, such as in mammalian expression systems. HaloTag® technology is based on the formation of a covalent bond between a protein fusion tag (HaloTag®) and synthetic chemical ligands. By interchanging different synthetic ligands researchers ultimately control the function and properties of the HaloTag® fusion protein. The HaloTag® system is designed to provide broad experimental flexibility and superb performance in both cell-based and biochemical assays such as protein purification, without switching fusion tags, or recloning. Available HaloTag® ligands include magnetic and nonmagnetic beads, glass slides, cell-permeable and impermeable fluorescent ligands, biotin, and reactive ligands to enable researchers to build their own custom HaloTag® ligands and surfaces. **(Figure 4.1)**.
For protein purification HaloTag® Technology is compatible with many protein expression systems and can be applied to proteins expressed in *E. coli*, mammalian cells and cell-free systems. HaloTag® protein is a good affinity tag for purification and also a good solubility tag. The lack of an endogenous equivalent of the HaloTag® protein in mammalian and most prokaryotic cells minimizes the chances of nonspecific interactions. The combination of covalent capture with rapid binding kinetics overcomes the equilibrium-based limitations associated with traditional affinity tags, and enables efficient capture even at low expression levels.

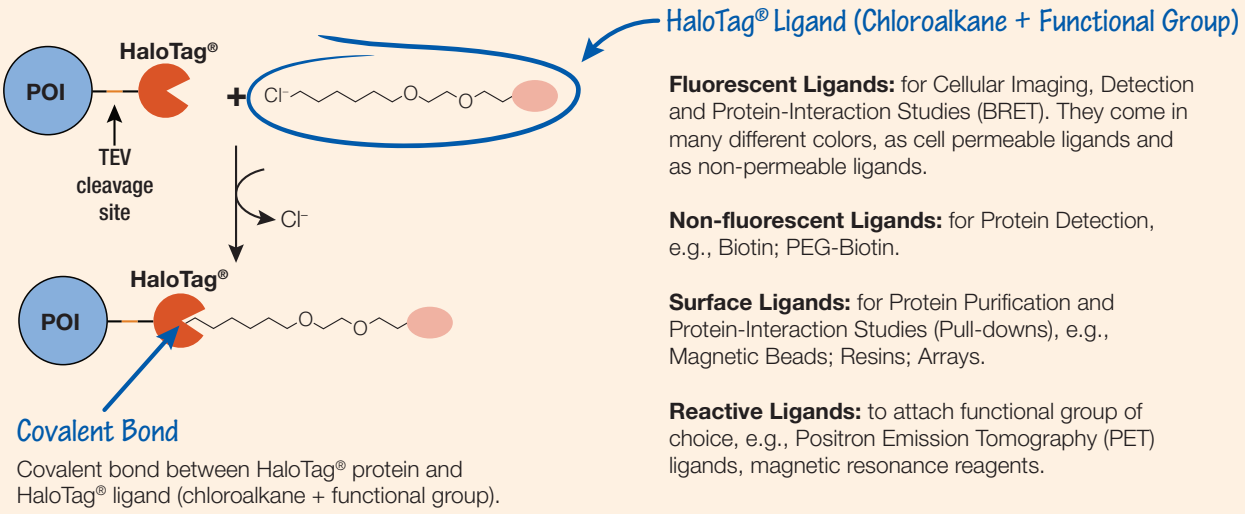


Figure 4.1. Schematic of HaloTag® technology consisting of the HaloTag® protein fused to a protein of interest (POI) and a selection of synthetic HaloTag® ligands carrying different functional groups. HaloTag® ligands specifically and covalently bind to the HaloTag® protein fusions.

HaloTag® Protein Purification System *(E. coli)*

Purification of HaloTag® fusion proteins from *E. coli* lysates.

Description

The HaloTag® Protein Purification System allows covalent, efficient and specific capture of proteins expressed in *E. coli* as HaloTag® fusion proteins. When a HaloTag® protein is fused to a target protein it functions as a robust solubility tag in addition to an affinity purification tag (note that solubility enhancement is achieved when a HaloTag® is fused to the N-terminus of the protein).The HaloTag® Protein Purification System allows stringent washing because of the covalent attachment of the HaloTag® fusion protein to the resin. ProTEV Protease cleaves the protein of interest from the HaloTag® protein, which is bound to the HaloLink™ Resin.

Principle

A protein of interest (POI) fused to a HaloTag® protein will covalently bind to the HaloLink™ Resin. The covalent nature of the linkage enables stringent and lengthy wash conditions without leaching the POI off the resin. After washing, the POI is eluted by proteolytic cleavage with ProTEV Protease. The ProTEV Protease contains a HQ-tag similiar to His-tag, allowing protease removal with the included HisLink™ resin.

Features and Benefits

- **Higher Solubility and Activity:** Compared to His-tag, GST and MBP affinity tags.
- **High Purity:** Covalent capture allows extensive and/or stringent washes without loss of bound protein, resulting in very low (<0.1%) nonspecific binding and a highly pure protein.
- **High Protein Recovery:** Rapid covalent capture; recovery is highly efficient > 75%.
- **High Yield:** >7mg of HaloTag® fusion protein per ml of HaloLink™ Resin.
- **Tag-Free Protein of Interest:** Due to proteolytic release coupled with protease removal.

Additional Information

Depending on the orientation of fusion constructs, 6 amino acids for N-terminal fusion constructs or 13 amino acids for C-terminal fusion constructs will remain on the POI after ProTEV cleavage.

References

Ohana, R.F. *et al.* (2009) HaloTag7: a genetically engineered tag that enhances bacterial expression of soluble proteins and improves protein purification. *Protein Expr Purif*. **68**(1), 110–20.

Neunuebel M.R. *et al.* (2012) Legionella pneumophila LidA affects nucleotide binding and activity of the host GTPase Rab1. *J Bacteriol.* **194**(6), 1389–400.

Verger, A. *et al.* (2013) The Mediator complex subunit MED25 is targeted by the N-terminal transactivation domain of the PEA3 group members. *Nucl. Acids Res.* **41**(9), 4847–59.

Ordering Information

HaloTag® Protein Purification System (Cat.# G6280)

HaloTag® Mammalian Protein Purification and Detection Systems

Quick purification of HaloTag® fusion proteins from mammalian cell culture lysates and supernatant.

Description

The HaloTag® Mammalian Protein Purification System is an optimized kit for rapid purification of HaloTag® fusion proteins from mammalian cell culture lysates and cell culture supernatants. An optimized TEV protease recognition site within the interconnecting polypeptide separates the HaloTag® protein and the fusion partner. The kit contains HaloLink™ Resin, HaloTEV Protease, 50X Protease Inhibitor Cocktail as well as Spin Columns. The covalent binding of HaloTag® fusion proteins, coupled with the low nonspecific binding of the HaloLink™ Resin, provides superior purity and recovery of recombinant proteins from cultured mammalian cells, even at low expression levels. The HaloTag® Mammalian Protein Detection and Purification System also contains a fluorescent ligand (TMRDirect™ Ligand) for the easy detection of HaloTag® fusion proteins by in-gel imaging, flow cytometry or microscopy applications. The simple-to-use fluorescent detection of HaloTag® fusion proteins facilitates rapid optimization of expression and purification conditions.

Principle

The protein of interest (POI), when fused to the HaloTag® protein, will covalently bind to the HaloLink™ Resin **(Figure 4.2)**. The covalent nature of the linkage enables stringent and lengthy wash conditions without concern of leaching the protein of interest off the resin. Post wash the POI is eluted by proteolytic cleavage with HaloTEV Protease. Since the HaloTEV Protease is fused to HaloTag® protein, the cleavage step and protease capture can be performed in a single step that separates the POI from the HaloTag fusion tag and the HaloTEV Protease.

Features and Benefits

- **High Purity:** Covalent capture allows extensive and/or stringent washes without loss of bound protein, resulting in very low (<0.1%) nonspecific binding and a highly pure protein.
- **High Protein Recovery:** Rapid covalent capture. Recovery is highly efficient, commonly >75%.
- **High Yield:** >7mg HaloTag® fusion protein per ml of HaloLink™ Resin.
- **Easily Scalable:** From 1ml up to 1L of mammalian cell culture.
- **Easy Detection:** Fluorescent HaloTag® ligands facilitate monitoring and optimization of the protein expression and purification procedure.

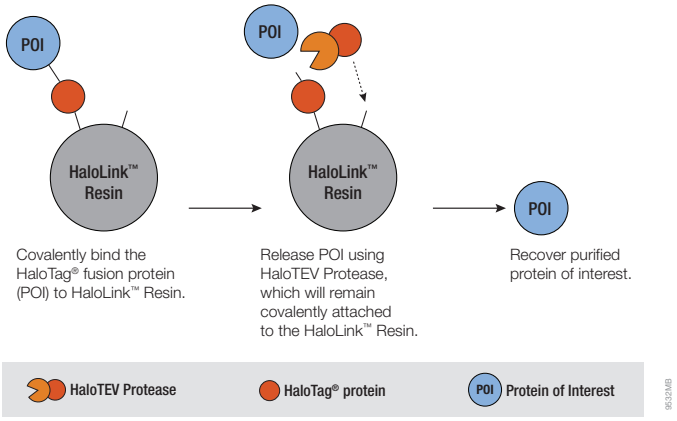


Figure 4.2. Schematic of the purification steps used with the HaloTag® Mammalian Protein Purification System.

Affinity-based Protein Purification

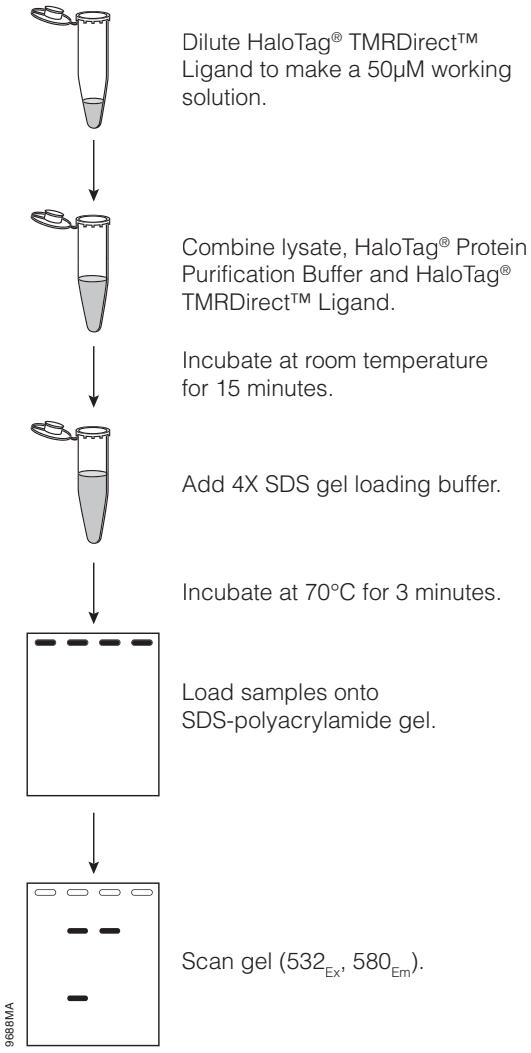


Figure 4.3 Schematic diagram of the fluorescent labeling of HaloTag® fusion protein with the HaloTag® TMRDirect™ Ligand.

Additional Information

Depending on the orientation of fusion constructs, 6 amino acids for N-terminal fusion constructs or 13 amino acids for C-terminal fusion constructs will remain on the POI after HaloTEV cleavage.

Fluorescent labeling of HaloTag® fusion proteins with the HaloTag® TMRDirect™ Ligand provides a rapid and convenient method to optimize protein expression and to monitor purification efficiency. HaloTag® protein fusions are briefly incubated with HaloTag® TMRDirect™ Ligand prior to SDS-PAGE **(Figure 4.3)**. After electrophoresis the proteins can be imaged without additional processing using a either a fluorescence gel scanner, such as GE Typhoon, or BIORAD ChemiDoc™ MP system.

References

Mammalian Cells

Ohana, R. *et al.* (2011) HaloTag-based purification of functional human kinases from mammalian cells. *Protein Expr. Purif.* **76**(2), 154–64.

Sun, X. *et al.* (2013) Hsp90 inhibitor 17-DMAG decreases expression of conserved herpesvirus protein kinases and reduces virus production in Epstein-Barr virus-infected cells. *J. Virol.* **87**(18), 10126–38.

Ryu MJ, (2012) Oncogenic Kras expression in postmitotic neurons leads to S100A8-S100A9 protein overexpression and gliosis. *J. Biol. Chem.* **287**(27), 22948–58.

Plant Cells

Lang, C. (2006) Purification from Plant. HaloTag: a new versatile reporter gene system in plant cells. *J. Exp. Bot.* **57**(12), 2985–92.

Yeast

Kardon, J.R. (2009) Regulation of the processivity and intracellular localization of Saccharomyces cerevisiae dynein by dynactin. *Proc. Natl. Acad. Sci. USA.* **106**(14), 5669–74.

Ordering Information

HaloTag® Mammalian Protein Purification System (Cat.# G6790)

HaloTag® Mammalian Protein Detection and Purification System (Cat.# G6795)

HisLink™ Protein Purification Resin

Purification of polyhistidine (His)-tagged proteins from bacterial and mammalian cells.

Description and Principle

HisLink™ Protein Purification Resin is a macroporous silica resin modified to contain a high level of tetradentate chelated nickel (>20mmol Ni/ml settled resin). The resin is designed to efficiently capture and purify overexpressed polyhistidine-tagged proteins. The HisLink™ Resin also may be used for general applications requiring an immobilized metal affinity chromatography (IMAC) matrix. The resin performs well in either column, batch or vacuum-based methods with a binding capacity of >15mg/ml of resin. The HisLink™ Protein Purification Resin is useful in all general immobilized metal affinity chromatography (IMAC) applications matrix as well as in low- to medium-pressure liquid chromatography systems. In batch format, HisLink™ Resin settles easily and may be separated from the lysate without filtration or centrifugation, simply by decanting. This enables rapid processing of larger quantities of lysate and the ability to purify protein without clearing the lysate of insoluble cellular debris.

Features and Benefits

- **Optimized Yields:** Binding capacity >15mg/ml.
- **Save Time:** Purify polyhistidine- or HQ-tagged proteins from cleared or crude cell lysates.
- **Flexible:** Use standard gravity column chromatography or automated applications such as FPLC.

Ordering Information

HisLink™ Protein Purification Resin
(Cat.# V8823, V8821)

References

Mochida, S. *et al.* (2010) A recombinant catalytic domain of matriptase induces detachment and apoptosis of small-intestinal epithelial IEC-6 cells cultured on laminin-coated surface. *J. Biochem.* **148**(6), 721-32.

Wei, L. *et al.* (2010) LPA19, a Psb27 homolog in Arabidopsis thaliana, facilitates D1 protein precursor processing during PSII biogenesis. *J. Biol. Chem.* **285**(28), 21391-8.

Herold, S. *et al.* (2009) Sab, a novel autotransporter of locus of enterocyte effacement-negative shiga-toxigenic Escherichia coli O113:H21, contributes to adherence and biofilm formation. *Infect. Immun.* **77**(8), 3234-43.

HisLink™ Spin Protein Purification System

Purification of polyhistidine (His)-tagged proteins in small volumes from bacterial cells.

Description

The HisLink™ Spin Protein Purification System provides a simple and fast system for purifying overexpressed His-tagged proteins from a 700µl sample of *E. coli* cell culture, using either a centrifuge- or vacuum-based method. The system contains cell lysis buffer, HisLink™ Resin, DNase I, Buffers, Collection Tubes and Spin Columns.

Principle

Protein can be purified directly from culture medium containing bacterial cells expressing a polyhistidine-tagged protein. The bacterial cells are lysed using FastBreak™ Cell Lysis Reagent, followed immediately by addition of HisLink™ Protein Purification Resin to the culture. Addition of these reagents results in simultaneous bacterial cell lysis and binding of the polyhistidine-tagged proteins. The samples then are transferred to a Spin Column where unbound protein is removed while the affinity resin is washed, and the target protein is recovered by elution. This system requires the use of a tabletop centrifuge or vacuum manifold. A schematic diagram of protein purification using the HisLink™ Spin System is shown in **Figure 4.4**.

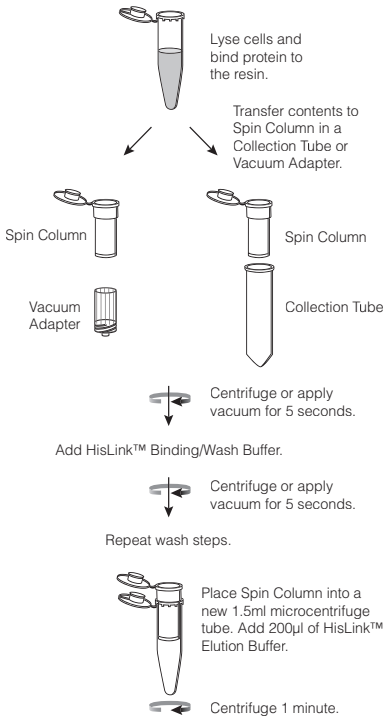


Figure 4.4. Schematic of polyhistidine-tagged protein purification using the HisLink™ Spin Protein Purification System.

Features and Benefits

- **Simple:** No cell culture preparation steps (no preclearing) required.
- **Quick:** No lengthy lysozyme incubations required to lyse cells.
- **Efficient:** Binding capacity of 1mg of polyhistidine-tagged protein per spin column.

Reference

Engel, L. *et al.* (2006) HisLink™ Spin Protein Purification System: Maximum Versatility in a Small Package. *Promega Notes* **93**, 2-4.

Ordering Information

HisLink™ Spin Protein Purification System
(Cat.# V1320)

HisLink™ 96 Protein Purification System

Purification of polyhistidine (His)-tagged proteins from bacterial and mammalian cells using a vacuum-based format.

Description

The HisLink™ 96 Protein Purification System provides a simple, and quick method of high-throughput purification of polyhistidine- or HQ-tagged overexpressed proteins from *E. coli* using a vacuum-based method. The system is designed to purify expressed polyhistidine-tagged proteins directly from deep-well 96-well culture plates. The HisLink™ System is amenable to manual or automated methods, such as the Beckman Coulter Biomek® 2000 or FX for high-throughput applications. The System contains cell lysis buffer, HisLink™ Resin, DNase I, Buffers and HisLink™ 96 Filtration & Collection Plates.

Principle

In preparation for protein purification, bacterial cells expressing a polyhistidine-tagged protein are lysed in culture using the provided FastBreak™ Cell Lysis Reagent. The HisLink™ Resin is added to the lysate and mixed; the polyhistidine-tagged proteins bind within a few minutes. Transfer the samples to a Filtration Plate, wash the resin to remove contaminants, and recover the target protein by elution **(Figure 4.5)**.

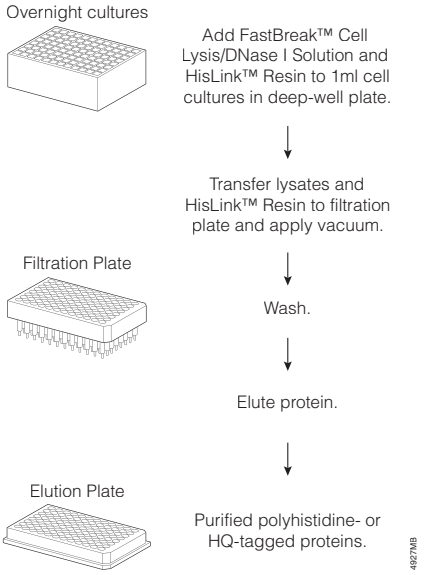


Figure 4.5. Flow diagram of polyhistidine-tagged protein purification using the HisLink™ 96 Protein Purification System.

Features and Benefits

- **Simple:** No centrifugation required—lysis buffer is added directly to cells in culture medium.
- **Quick:** No long lysozyme incubations are required for cell lysis.
- **Versatile:** Perform purification manually or on an automated platform.
- **Efficient:** Binding capacity of 1mg of polyhistidine-tagged protein per well.

Additional Information

Note: This system requires the use of the Vac-Man® 96 Vacuum Manifold or compatible vacuum manifold.

Reference

Engel, L. *et al.* (2005) HisLink™ 96 Protein Purification System: Fast Purification of Polyhistidine-Tagged Proteins. *Promega Notes* **90**, 15–18.

Ordering Information

HisLink™ 96 Protein Purification System
(Cat.# V3680, V3681)

SoftLink™ Soft Release Avidin Resin

Reversible binding and purification of biotinylated proteins under mild nondenaturing conditions, purification of biotinylated antibodies and other proteins as well as in vivo biotinylated fusion proteins from PinPoint™ Vectors.

Description

SoftLink™ Avidin Resin can be used for the isolation and purification of biotinylated molecules. SoftLink™ Resin is a rigid, methacrylate polymeric gel filtration matrix, functionalized with a covalently bound, monomeric avidin that does not leach under elution conditions. The monomeric avidin enables purification of biotinylated proteins under mild elution conditions due to a lower K_d than tetrameric biotin (10^{-7}M vs. 10^{-15}M). Monomeric avidin allows specific capture and gentle elution that preserves the integrity of sensitive proteins.

Features and Benefits

- **Sensitive:** Binds 20–40nmol of biotinylated protein per 1ml of settled resin. Up to 4mg of biotinylated protein has been purified per ml resin.
- **Gentle:** Elution of biotinylated proteins under mild nondenaturing conditions (5mM biotin).
- **Reusable:** Regenerates at least 10 times without loss of binding capacity.
- **Flexible:** Purification by batch or column method, in both mild and stringent conditions such as pH 2-13, ionic strength up to 2M NaCl, denaturing conditions (6M guanidine, 1% SDS).

Ordering Information

SoftLink™ Avidin Resin
(Cat.# V2011, V2012)

References

Hoke, D.E. *et al.* (2008) LipL32 is an extracellular matrix-interacting protein of Leptospira spp. and Pseudoalteromonas tunicata. *Infect. Immun.* **76**(5), 2063–9.

Field, S. *et al.* (2008)The 'zinc knuckle' motif of Early B cell Factor is required for transcriptional activation of B cell-specific genes. *Mol. Immunol.* **45**(14), 3786–96.

Zamft, B. *et al.* (2012) Nascent RNA structure modulates the transcriptional dynamics of RNA polymerases. *Proc. Natl. Acad. Sci. USA* **109**(23), 8948–53.

PinPoint™ Xa Protein Purification System

Production and purification of fusion proteins that are biotinylated in *E. coli*.

Description and Principle

The PinPoint™ Xa Protein Purification System is designed for the production and purification of fusion proteins that are biotinylated at the N-terminus in vivo. The DNA coding for the protein of interest is cloned into a PinPoint™ Vector downstream of a sequence encoding a 14kDa peptide that becomes biotinylated in vivo. Biotinylated fusion proteins are produced in *E. coli* (JM109 and HB101 *E. coli* bacterial strains, or other strains without the birA mutation) and are affinity-purified using the SoftLink™ Soft Release Avidin Resin. The PinPoint™ Vectors feature the encoded endoproteinase Factor Xa, for proteolytic removal of the biotinylated peptide post-purification.

The system contains 3 cloning vectors in multiple combinations of sense reading frames, an avidin-conjugated resin, Streptavidin-Alkaline Phosphatase, a purification column and biotin. The PinPoint™ Xa Control Vector contains the chloramphenicol acetyl-transferase (CAT) gene and is provided as a means of monitoring protein expression, purification and processing conditions. The system generally yields 1–5mg of protein per liter of *E. coli* culture.

Features and Benefits

- **In vivo Biotinylation Tag:** Allows purification of fusion proteins labeled with biotin.
- **Easy-to-Use:** Purification of biotinylated proteins with the SoftLink™ Resin can be performed by column or batch purification.
- **Flexible:** PinPoint™ Vectors are supplied for all reading frames.
- **Gentle Release Conditions:** SoftLink™ Resin allows release of the fusion protein under nondenaturing conditions.

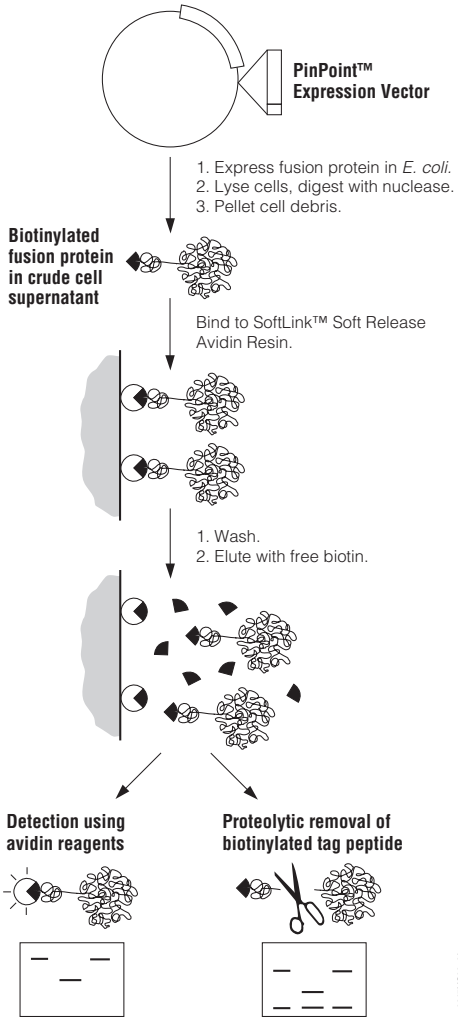


Figure 4.6. Schematic diagram of recombinant protein expression and purification using the PinPoint™ Xa Protein Purification System.

Ordering Information

PinPoint™ Xa Protein Purification System (Cat.# V2020)

4.2 Magnetic Affinity-based Purification and Pull-down Strategies

OVERVIEW

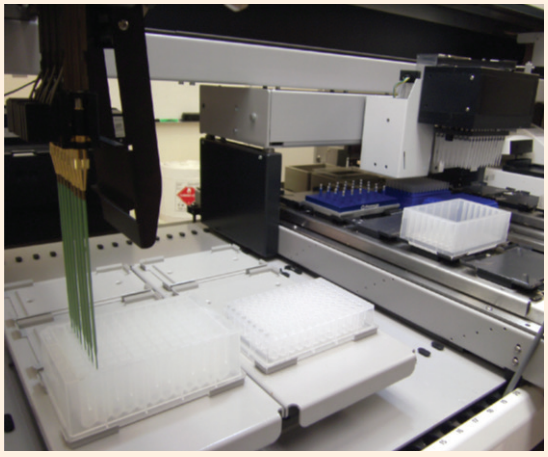
The use of magnetic beads for protein purification/pull-down provides a rapid and efficient method to extract and capture recombinant proteins from cell lysates and cell culture supernatant. Virtually all affinity chemistries discussed previously can be transferred on magnetic beads. There are many advantages of a magnetic approach:

1. Easily process very small sample volumes. Proteins from volumes as low as 20µl can be processed with good efficiency and minimal target protein losses.
2. Simple and cost effective approach that does not require extensive plumbing, chromatography instrumentation or centrifugation.
3. Protein samples can be eluted into a minimal volume resulting in higher concentration of purified protein. This can be used for protein concentration purposes.
4. Faster washing and elution steps because no centrifugation steps are required.
5. More readily adapted for high-throughput applications on automated liquid-handling robots **(Figure 4.7).**
6. Magnetic approach excels at parallel sample processing, where multiple samples need to be processed as fast as possible.

All previously discussed affinity chemistries (His, HaloTag® GST) are available for protein capture in a magnetic bead format and are amenable to both protein purification and protein pull-down applications.

A.

B.



10543TB

Figure 4.7. Magnetized beads in an Eppendorf tube during protein purification from small (µl) sample volumes **(Panel A)**. Tecan automated liquid handler processing magnetic beads during high-throughput purification **(Panel B)**.

MagneGST™ Protein Purification System

Manual or automated magnetic purification or pull-down of glutathione-S-transferase (GST) fusion proteins from crude or cleared *E. coli* lysates.

Description

The MagneGST™ Protein Purification System includes immobilized glutathione paramagnetic particles, buffers and cell lysis reagents to isolate GST-tagged protein directly from a crude or cleared lysate. GST-tagged proteins can be purified from 1ml to 50ml of culture. Samples also can be processed using a robotic platform. MagneGST™ particles are supplied as a 25% slurry and have a binding capacity of 5–10mg of GST protein per 1ml of settled resin.

Principle

Bacterial cells containing a GST-fusion protein are lysed using the provided MagneGST™ Cell Lysis Reagent or with an alternative lysis method, then the MagneGST™ particles are added directly to the crude lysate. GST-fusion proteins bind to the MagneGST™ particles. Unbound proteins are washed away, and the GST-fusion target protein is recovered by elution with 50mM glutathione **(Figure 4.8)**.

For capture and verification of interacting proteins (pull-down), the GST-tagged bait protein is expressed in *E. coli* and the potential prey proteins are expressed in cell-free system. The bait is bound on the MagneGST™ particles and is then used to capture different prey proteins from cell-free expression reactions **(Figure 4.8).**

Features and Benefits

- **Simple:** One-step purification of multiple samples with easy handling. No lysate clearing needed.
- **Scalable Protocol:** Use 1–50ml of cell culture. Obtain 5–10mg of GST protein per 1ml of settled beads.
- **Efficient:** Achieve high yields with little or no nonspecific background.

References

Meloni, A. *et al.* (2010) DAXX is a new AIRE-interacting protein. *J. Biol. Chem.* **285**(17), 13012–21.

Maier R.H, *et al.* (2010) Epitope mapping of antibodies using a cell array-based polypeptide library. *J. Biomol. Screen.* **15**(4), 418–26.

Morimoto, H. *et al.* (2008) Procollagen C-proteinase enhancer-1 (PCPE-1) interacts with beta2-microglobulin (beta2-m) and may help initiate beta2-m amyloid fibril formation in connective tissues. *Matrix Biol.* **27**(3), 211–9.

Ordering Information

MagneGST™ Protein Purification System (Cat.# V8600, V8603)

Magnetic Affinity-based Protein Purification

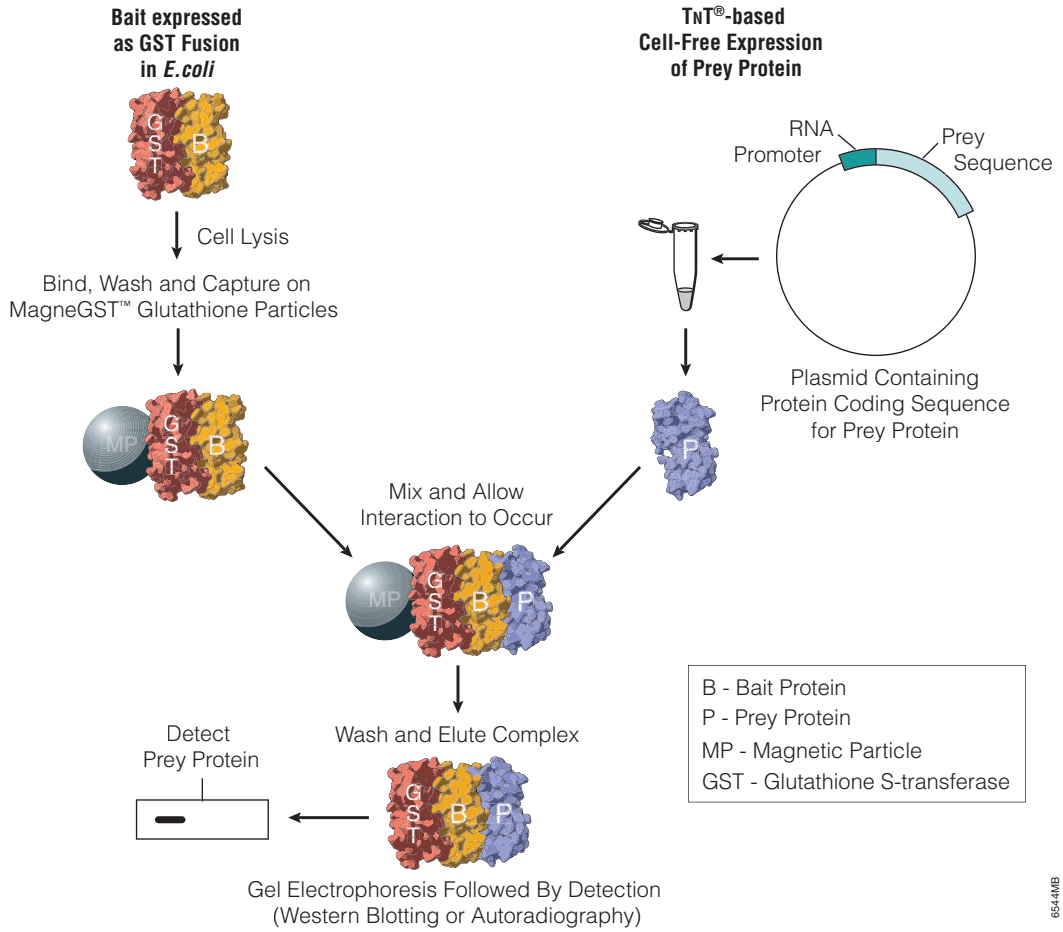


Figure 4.8. Schematic diagram of MagneGST™ Protein Purification in combination with protein pull-down.

Magne™ HaloTag® Beads

Manual or automated high-throughput protein purification and pull-down of HaloTag® fusion proteins from *E. coli* and mammalian cell cultures, at input volumes from 20µl to 50ml.

Description and Principle

The Magne™ HaloTag® Beads provide a convenient method to covalently capture HaloTag® fusion proteins with magnetic particles for protein pull-downs and purification. HaloTag® fusion proteins may be expressed in cell-based and cell-free systems. Cell lysates are mixed with Magne™ HaloTag® Beads and HaloTag® fusion proteins bound covalently to the beads. These magnetic beads offer a high-binding capacity (≥20mg/ml) for purifying HaloTag® fusion proteins with low nonspecific protein binding. After washing, the protein of interest can be released from the beads by TEV Protease cleavage (either HaloTEV or ProTEV) at the optimized TEV recognition site. Proteolytic release yields the protein of interest, while the HaloTag® protein and HaloTEV Protease remain covalently attached to the beads **(Figure 4.9)**.

Features and Benefits

- **Simple:** No centrifugation or vacuum is required once the cells are lysed.
- **High Recovery:** Binding capacity ≥20mg of purified HaloTag® fusion protein per ml of settled particles.

References

Verger A. *et.al.* (2013) The Mediator complex subunit MED25 is targeted by the N-terminal transactivation domain of the PEA3 group members. *Nucl. Acids Res.* **41**(9), 4847–59.

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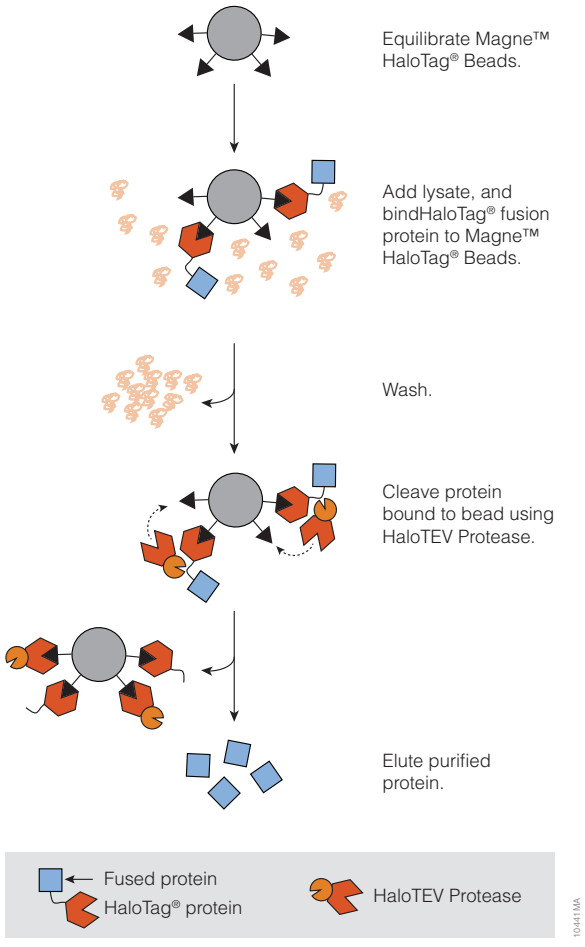

Figure 4.9 Schematic diagram of the HaloTag® fusion purification protocol using Magne™ HaloTag® Beads.

Ordering Information

Magne™ HaloTag® Beads
(Cat.# G7281, G7282)

MagneHis™ Protein Purification System

Manual or automated high-throughput protein purification and pull-down of polyhistidine-tagged proteins, from crude *E. coli* cell lysates, insect or mammalian cell lysates or cell supernatant.

Description and Principle

The MagneHis™ Protein Purification System provides a quick and simple method for the purification of polyhistidine-tagged, overexpressed proteins. Paramagnetic precharged nickel particles (MagneHis™ Ni-Particles) are used to isolate His-tagged fusion proteins directly from a crude cell lysate. Sample volumes from 20µl to 50ml can be easily processed manually. Samples can also be processed using a robotic platform such as the Beckman Coulter Biomek® 2000 or FX or Tecan Genesis® RSP. This Purification System contains magnetic beads and buffers as well as FastBreak™ Cell Lysis Reagent. His tagged proteins can be expressed either intracellularly or secreted into cell media. Bacterial, insect or mammalian cells can be lysed with FastBreak™ Cell Lysis Reagent or with other lysis methods. MagneHis™ Ni-Particles are then added to the lysate and bind to His tag containing proteins. Unbound proteins are washed away, and the target protein is recovered by elution with imidazole **(Figure 4.10)**. Purification can be performed under native and denaturing conditions.

Features and Benefits

- **Fast:** No long incubations with lysozyme are required for cell lysis.
- **Simple:** No centrifugation or vacuum after cell lysis.
- **Efficient:** Binding capacity of up to 1mg of polyhistidine-tagged protein per 1ml of MagneHis™ Ni-particles.
- **Versatile:** Purify His-tagged proteins in manual format or at high-throughput with an automated liquid handler.

References

Guo, H. *et al.* (2010) Osteopontin and protein kinase C regulate PDLIM2 activation and STAT1 ubiquitination in LPS-treated murine macrophages. *J Biol Chem.* **285**(48), 37787–96.

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Koschubs, T. *et al.* (2010) Preparation and topology of the Mediator middle module. *Nucleic Acids Res.* **38**(10), 3186–95.

Ordering Information

MagneHis™ Protein Purification System
(Cat.# V8500, V8550)

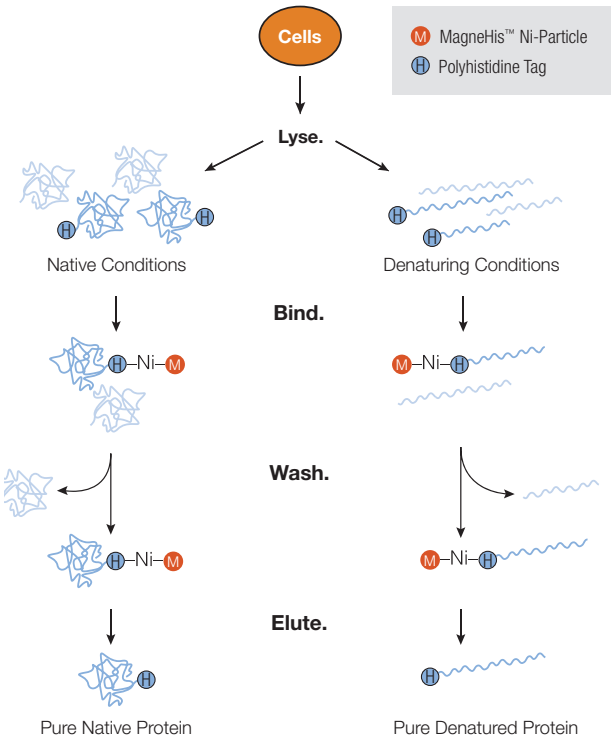


Figure 4.10. Schematic of the MagneHis™ Protein Purification System protocol.

Maxwell® 16 Polyhistidine Protein Purification Kit

Automated purification of polyhistidine-tagged proteins from bacterial cultures and mammalian and insect proteins.

Description and Principle

The Maxwell® 16 Polyhistidine Protein Purification Kit consists of cartridges prefilled with MagneHis™ Ni-Particles and buffers that are used with the Maxwell® 16 Instrument to provide an easy method for efficient, automated purification of polyhistidine-tagged protein from various cells **(Table 4.2)**. The Maxwell® Instrument is supplied with a preprogrammed purification procedure and reagent cartridges specifically designed to maximize simplicity and convenience. The instrument can process up to 16 samples in approximately 40 minutes.

Features and Benefits

- **Save Hands-On Time:** Prefilled cartridges eliminate reagent preparation, multiple pipetting steps, centrifugation and additional sample manipulation.
- **Consistent Results:** Across all samples.

Table 4.2. Sample Types and Maximum Processing Capacity per Cartridge.

Sample Type	Processing Capacity
Bacterial Culture	Up to 20 O.D.$_{600}$
Mammalian Cell Culture Cells	Up to 5 x 10^6 cells
Insect Cell Culture Cells	Up to 5 x 10^6 cells
Mammalian or Insect Cell Culture Medium	1ml

Figure 4.11. The Maxwell® 16 Instrument uses paramagnetic-particle technology to extract DNA, RNA or protein from up to 16 samples in less than 45 minutes. The paramagnetic particles and reagents are supplied in prefilled cartridges for faster separations with less hands-on time.

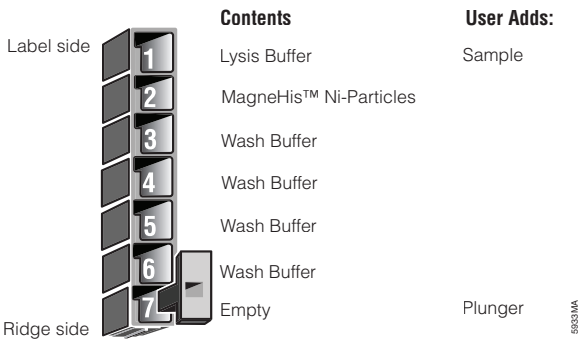


Figure 4.12. Maxwell 16® Polyhistidine Protein Purification Sample Cartridge

Ordering Information

Maxwell® 16 Polyhistidine Protein Purification Kit (Cat.# AS1060)

Magnetic Separation Devices

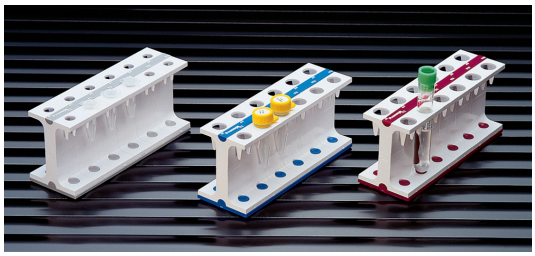
Manual or automated protein purification using magnetic particles.

Description

Promega offers a wide range of magnetic devices for separations from 0.5ml microcentrifuge tubes to 15ml or 50ml conical tubes, to 96- and 384-well standard and deep-well plates. The magnetic separation device for plates is useful for both manual and automated liquid-handling.

MagneSphere® Technology Magnetic Separation Stands

MagneSphere® Technology Magnetic Separation Stand (two-position). Up to two sample volumes (50µl –1.0ml). Left to Right: Cat.# **Z5331, Z5332, Z5333**.



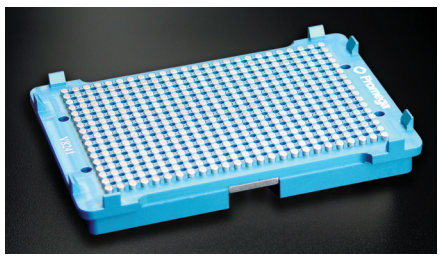
MagneSphere® Technology Magnetic Separation Stand (twelve-position). Up to two sample volumes (50µl–1.0ml). Left to Right: Cat.# **Z5341, Z5342, Z5343**.

PolyATtract® System 1000 Magnetic Separation Stand. One Sample volume (1-50ml). Cat.# **Z5410**.

MagnaBot® Magnetic Separation Devices

MagnaBot® 96 Magnetic Separation Device for 96-well standard- or deep-well-plates (20µl– 1.0ml). Cat.# **V8151**.

MagnaBot® II Magnetic Separation Device for 96-well PCR plate. Cat.# **V8351**.



MagnaBot® 384 Magnetic Separation Device. Cat.# **V8241**.