Maxwell® 16 Polyhistidine Protein Purification Kit: Automated Protein Purification with Maximum Performance and Convenience

ABSTRACT The Maxwell® 16 Polyhistidine Protein Purification Kit allows simple, convenient, and robust purification of polyhistidine-tagged proteins from multiple sample types, including bacteria, mammalian cells, insect cells, and culture medium. The system also purifies HQ-tagged proteins from bacterial samples. The optimized automated method, compact instrumentation, and pre-filled reagent cartridges maximize performance while minimizing the hands-on time required for protein purification. The purified protein is compatible with many common downstream applications including polyacrylamide gel electrophoresis and detection, functionality studies, Western blot analysis, and mass spectrometry. In this article we demonstrate the performance of the Maxwell® 16 Polyhistidine Protein Purification Kit for low- to moderate-throughput automated protein purification. Purification results with the Maxwell® System are comparable to or better than those achieved using manual processing methods.

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Maxwell® 16
Instrument provides high-quality purified polyhistidine- and HQ-tagged proteins in a low- to moderate-throughput approach.

INTRODUCTION

The Maxwell® 16 Polyhistidine Protein Purification Kit[a,b] uses MagneHis™ Ni-Particles for the automated purification of polyhistidine- or HQ-tagged recombinant proteins, increasing sample processing capacity and reducing hands-on time. The low- to moderate-throughput and compact instrumentation are ideal for protein purification for clone expression optimization, multiple target cell expression screening, functionality testing, and structural analysis.

Production and purification of recombinant proteins using bacterial, mammalian, and insect expression systems are increasingly common techniques, particularly with the emerging emphasis on proteomics. Proteins expressed and purified from these systems are used in many applications, including enzymatic assays, investigation of intermolecular interactions, and structural studies. Most recombinant proteins contain a fusion partner or affinity tag to facilitate purification and detection (1), the most common being the polyhistidine tag, which contains 5–10 histidine residues at either the C- or N-terminus of the expressed protein. Advantages of the polyhistidine tag are that it adds only 0.84kDa to the mass of the protein and is nonimmunogenic. Also, because the tertiary structure of the tag is not important for purification, polyhistidine-tagged proteins can be purified using native or denaturing conditions.

The affinity of histidine residues for immobilized nickel allows selective purification of polyhistidine-tagged proteins (2,3), as well as HQ-tagged proteins (4). The HQ tag consists of 6 amino acid residues (HQHQHQ; H=histidine, Q=glutamine) and shares similar features with the polyhistidine tag. However, the HQ tag may elute from affinity columns at lower imidazole concentrations, potentially making it more useful for applications such as enzymatic reactions. MagneHis™ Ni-Particles allow the efficient purification of either polyhistidine- or HQ-tagged proteins due to maximal binding of the target protein to the nickel substrate and minimal nonspecific binding.

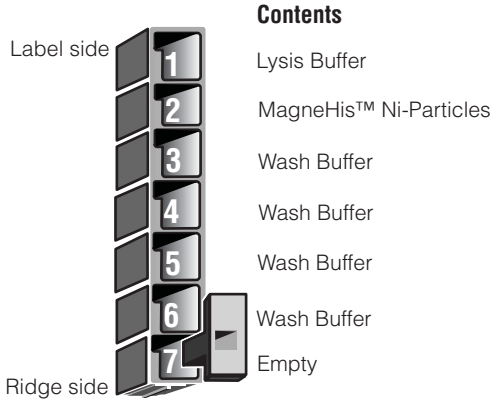

Figure 1. Maxwell® 16 Polyhistidine Protein Purification Kit reagent cartridge.

AUTOMATED PROTEIN PURIFICATION WITH PREFILLED REAGENT CARTRIDGES

The Maxwell® 16 Polyhistidine Protein Purification Kit is designed for purification of polyhistidine- or HQ-tagged proteins with little hands-on time and labor, while providing high yield and purity of the recombinant protein. The pre-filled reagent cartridges contain everything required for successful protein purification: lysis buffer, MagneHis™ magnetic Ni-Particles and Wash Buffer (Figure 1). Elution Tubes, Elution Buffer and plungers for use with the Maxwell® 16 Instrument are also provided. Thus no reagent preparation is required.

The Maxwell® 16 Instrument contains an optimized protocol for easy-to-perform protein purification and can process 1–16 samples in about 40 minutes. The instrument, which is small and easy to operate (5), uses a powerful magnet and unique plunger design to lyse and capture the target material while washing away impurities. The sample is added to well #1 of the reagent cartridge, which is then placed into the instrument. Simple on-screen prompts guide you through selection of the protein purification method. Prior to initiation of the purification program, Elution Tubes are also loaded into the machine and filled with 300µl of Elution Buffer per tube.

To demonstrate the robust nature of the purification process and the consistency between individual samples within a run and between runs, a bacterial culture expressing polyhistidine-tagged firefly luciferase was processed in replicates of 16 in two separate purification runs. Figure 2 shows that the difference in protein purification within a run and between runs was very small (~3%), demonstrating the robust nature of the reagents and the automated method.

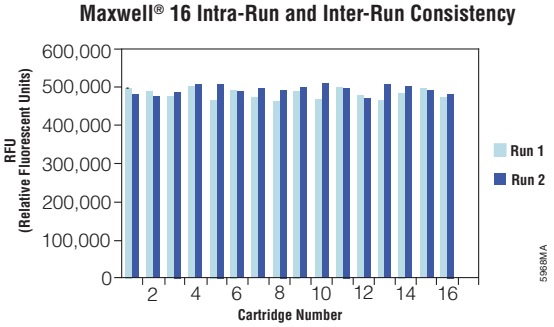

Figure 2. Intra-run and inter-run consistency of luciferase purification using the Maxwell® 16 Polyhistidine Protein Purification Kit and the Maxwell® 16 Instrument. Polyhistidine-tagged firefly luciferase was expressed in bacterial strain BL21(DE3) and purified according to the Maxwell® 16 System protocol (Technical Manual #TM285). Yields were determined by gel analysis followed by SYPRO®-Ruby fluorescent staining.

COMPATIBILITY WITH DIFFERENT SAMPLE TYPES

Expression and purification of recombinant proteins is often performed from multiple sample types to increase the likelihood of obtaining functional protein. We used the Maxwell® 16 Polyhistidine Protein Purification Kit to purify polyhistidine-tagged proteins from various samples including bacterial cultures, mammalian cells, insect cells, and culture medium. The system can process up to 20 O.D.$_{600}$ of bacterial culture, 5 × 10^6 mammalian or insect cells, or up to 1ml of culture medium. Bacterial cultures may be grown in LB, TB, or CIRCLEGROW® media, and protein purification can be performed directly from cells in media without prior processing. The addition of DNase to samples is recommended when processing bacterial cultures with an O.D.$_{600}$ of 4 or greater, or >2 × 10^6 mammalian or insect cells. For mammalian and insect cells and culture media containing serum, the addition of 200–500mM NaCl to the wells containing wash buffer can decrease nonspecific binding.

To demonstrate purification from bacterial cultures, various polyhistidine- or HQ-tagged proteins were purified from cultures expressing polyhistidine- or HQ-tagged clones. For each sample, 20 O.D.$_{600}$ was used according to the protocol provided in the *Maxwell® 16 Polyhistidine Protein Purification Kit Technical Manual* #TM285. The purified proteins were analyzed by polyacrylamide gel electrophoresis followed by Coomassie® blue staining. Seven different polyhistidine-tagged proteins were successfully purified from bacterial strains BL21(DE3) and BL21(DE3)pLysS (Figure 3A), and six different HQ-tagged proteins were successfully purified from bacterial strains BL21(DE3) and Single Step (KRX) (6; Figure 3B).

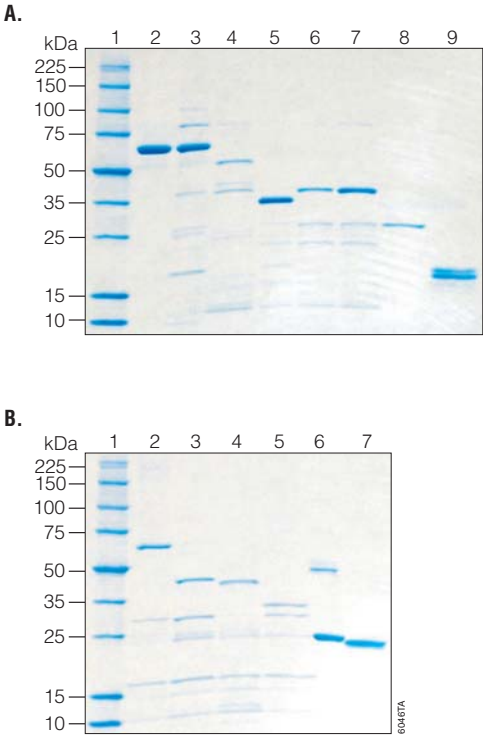

Figure 3. Purification of polyhistidine- and HQ-tagged proteins from various sample types. All proteins were purified from 20 O.D.$_{600}$ of culture using the Maxwell® 16 Polyhistidine Protein Purification Kit. Unless otherwise indicated, proteins were purified from bacterial strain BL21(DE3). **Panel A.** Polyhistidine-tagged proteins. Lane 1, 5µl Broad Range Protein Molecular Weight Marker (Cat.# V8491); lane 2, 2.5µl His-Luciferase (~62kDa); lane 3, 8µl His-Luciferase (~62kDa) from BL21(DE3)pLysS; lane 4, 20µl His-MAPK (~52kDa); lane 5, 15µl His-*Renilla* (~36kDa); lane 6, 20µl His-Actin (~35kDa); lane 7, 20µl His-Calmodulin (~31kDa); lane 8, 20µl His-GFP (~27kDa); lane 9, 10µl His-Id (~18kDa). **Panel B.** Purified HQ-tagged proteins. Lane 1, 5µl Broad Range Molecular Weight Marker (Cat.# V8491); lane 2, 10µl HQ-Luciferase (~62kDa); lane 3, 17µl HQ-MBP (~45kDa); lane 4, 19µl HQ-PKA(cat) (~44kDa); lane 5, 19µl HQ-*Renilla* from Single Step (KRX) Cells (~36kDa); lane 6, 2µl GST-HQ (~25kDa); lane 7, 1.5µl HQ-CAT (~24kDa).

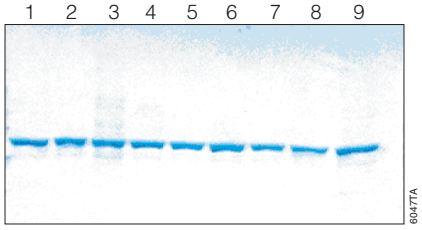

Figure 4. Purification of spiked polyhistidine-tagged luciferase from cells (2 × 10⁶ in 1ml) and culture media (1ml). Lanes 1 and 9, input His-Luciferase; lane 2, CHO cells; lane 3, Sf9 cells; lane 4, DMEM + FBS; lane 5, DMEM; lane 6, F12; lane 7, RPMI-1640; lane 8, BacVector® insect medium. BacVector® medium was obtained from BD Bioscience; all other media were obtained from Invitrogen Corporation.

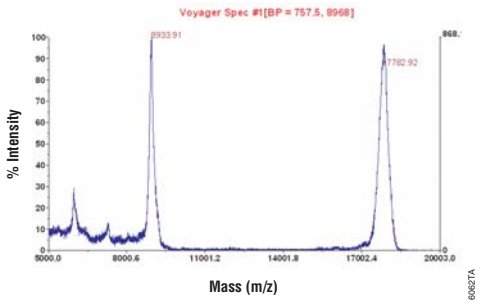

Figure 5. MALDI-TOF analysis of His-Id eluted from the MagneHis™ Ni-Particles in the Maxwell® 16 Polyhistidine Protein Purification Kit using 70% ACN/0.1% TFA. His-Id is ~18kDa and is the large peak the farthest to the right.

Purified protein is compatible with downstream applications including polyacrylamide gel electrophoresis, Western blot analysis and mass spectrometry.

For His-luciferase and for HQ-CAT, approximately 300µg of protein were obtained from single 20O.D.$_{600}$ samples—much more than obtained with comparable manual purification methods (data not shown).

Purification from a mammalian cell line (Chinese Hamster Ovary), an insect cell line (Sf9), and various types of culture media was performed by measuring purification of exogenously supplied polyhistidine-tagged luciferase from the different cell types in comparison to the amount of input protein. The results are shown in Figure 4 and demonstrate successful purification of polyhistidine-tagged protein from 2 × 10⁶ CHO cells, 2 × 10⁶ Sf9 cells, 1ml of DMEM medium with or without fetal bovine serum, F12 medium, RPMI-1640 medium, and BacVector® insect cell medium. The addition of 500mM sodium chloride to the wash wells reduced any nonspecific binding, which can occur with some of these sample types. In addition, the Maxwell® 16 Polyhistidine Protein Purification Kit may be used for purification of polyhistidine- and HQ-tagged proteins from in vitro expression systems such as the TnT® SP6 High-Yield System (data not shown).

PURIFIED PROTEIN IS READY FOR DOWNSTREAM APPLICATIONS

To verify that the purified protein was compatible with multiple downstream applications, His-Id was purified from 20 O.D.$_{600}$ of bacterial culture and then eluted into 300µl 70% acetonitrile/0.1% trifluoroacetic acid. The sample was then dried under vacuum, resuspended in 30µl of 0.1% TFA and purified using C18 ZipTip® columns (Millipore). The bound material was eluted in 50% acetonitrile/0.1%TFA containing 10mg/ml CHCA matrix, spotted directly onto a MALDI-TOF target and analyzed by mass spectrometry using an Applied Biosystems Voyager™ 1012 instrument. The protein purified with the Maxwell® 16 Polyhistidine Protein Purification Kit and the Maxwell® 16 Instrument was compatible with this type of analysis (Figure 5). The His-Id protein, which is approximately 18kDa, can be identified as the strong peak at 17782.92 molecular weight.

Flexible
The Maxwell® 16 Instrument can be used to purify polyhistidine- and HQ-tagged protein as well as genomic DNA, RNA and DNA for forensic applications.

Purification of polyhistidine-tagged proteins from mammalian cells was also analyzed using CHO cells transfected with expression vectors encoding His-MAPK9 (Mitogen Activated Protein Kinase 9) or His-CAM1 (Calmodulin 1). Protein was purified from cells 48 hours post transfection and also from transfected cells that were selected with G-418 for up to 23 days. The Maxwell® 16-purified protein samples were then analyzed by Western blot using an anti-penta-His antibody and an HRP-conjugated secondary antibody followed by chemiluminescent detection. A sample of untransfected cells was processed as a negative control. Both His-MAPK9 and His-CAM1 were purified successfully from 48-hour and 23-day CHO cells (Figure 6). A background band of around 70kDa was present in all samples, including untransfected controls. This background protein band probably contains a series of histidine residues that cause binding to the MagneHis™ Ni-Particles. The His-MAPK9 is visible after 23 days of selection. The His-CAM1 was not visible after selection (data not shown). Thus, the Maxwell® 16 Instrument and the Maxwell® 16 Polyhistidine Protein Purification Kit were shown to be compatible with purification of endogenously expressed polyhistidine-tagged proteins from mammalian cells.

To investigate the functionality of proteins purified using the Maxwell® 16 Polyhistidine Protein Purification Kit, an activity assay was performed using HQ-PKA (Protein Kinase A catalytic subunit) purified from Single Step (KRX) bacterial cells. Kinase activity of increasing amounts of purified HQ-PKA and Kemptide was determined using the Kinase-Glo® Assay (Cat.# V6711). The results in Figure 7 demonstrate that, as the amount of HQ-PKA increases, signal from the luciferase reporter reaction decreases. This occurs as ATP is utilized in the kinase reaction and is no longer available for the luciferase reaction (7). Thus, the HQ-PKA purified using the Maxwell® 16 System was shown to be functional. We have also demonstrated the functionality of polyhistidine- and HQ-tagged firefly and *Renilla* luciferase proteins purified using the Maxwell® 16 System (data not shown).

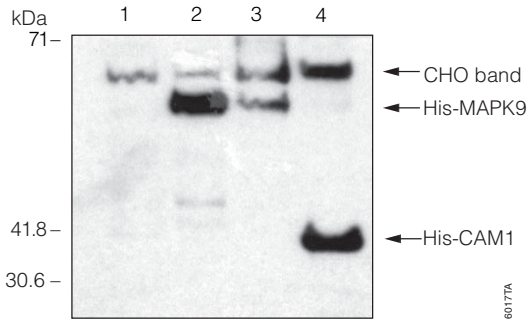

Figure 6. Western blot analysis of His-MAPK9 or His-CAM1 purified from CHO cells using the Maxwell® 16 Polyhistidine Protein Purification Kit. Aliquots (15µl) of each purified protein were separated on a 4–12% Bis-Tris gel with MES/SDS running buffer. The samples were then transferred to Hybond®-C membrane and developed using anti-penta His mAb (1:1,000; Qiagen) and anti-mouse IgG-HRP conjugate (1:2,500; Cat.# W4021) followed by chemiluminescent detection (Transcend™ System, Cat.# L5080). Exposure was for 2 minutes on Kodak X-OMAT® film. Lane 1, untransfected control; lane 2, CHO cells transfected for 2 days with CMV-His-MAPK9 plasmid DNA; lane 3, CHO cells transfected with CMV-His-MAPK9 and selected for 23 days with G-A418; lane 4, CHO cells transfected for 2 days with CMV-His-CAM1.

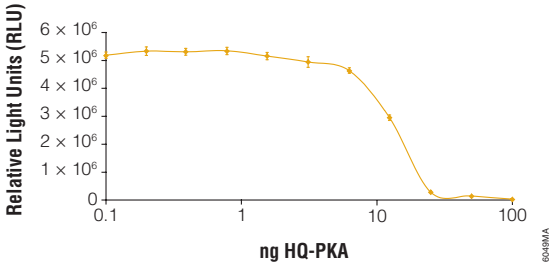

Figure 7. Kinase activity of HQ-PKA purified using the Maxwell® 16 Polyhistidine Protein Purification Kit. Increasing amounts of purified HQ-PKA were incubated with Kemptide and ATP in kinase buffer. After 20 minutes at room temperature, Kinase-Glo® Reagent was added and light output measured on an EG&G Berthold plate-reading luminometer. The results are the average of triplicate reactions at each time point.

CONCLUSIONS

The Maxwell® 16 Polyhistidine Protein Purification Kit provides labor savings and ease-of-use for low- to moderate-throughput purification of polyhistidine- and HQ-tagged proteins from multiple sample types. Purification is robust and consistent and provides yields and purity comparable to or better than those achieved using manual purification methods. The Maxwell® 16 System also provides the convenience of pre-filled reagent cartridges and uses a compact and simple instrument that requires minimal training and maintenance. The Maxwell® 16 Instrument provides maximum flexibility, as it can also be used for purification of genomic DNA (5) and RNA (8) and for purification of DNA for forensic applications (9).

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PROTOCOLS

- *Maxwell® 16 Instrument Operating Manual* #TM274, Promega Corporation.
  www.promega.com/tbs/tm274/tm274.html
- *Maxwell® 16 Polyhistidine Protein Purification System Technical Manual* #TM285, Promega Corporation.
  www.promega.com/tbs/tm285/tm285.html

ORDERING INFORMATION

Product	Size	Cat.#
Maxwell® 16 Instrument*	1 each	AS1000
Maxwell® 16 Polyhistidine Protein Purification Kit	48 preps	AS1060

*For Research Use Only. Not for use in diagnostic procedures.

Related Products

Product	Size	Cat.#
MagneHis™ Protein Purification System	65 reactions	V8500
	325 reactions	V8550
MagneHis™ Ni-Particles	2ml	V8560
	10ml	V8565
FastBreak™ Cell Lysis Reagent, 10X	15ml	V8571
	60ml	V8572
	100ml	V8573

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