



Promega

Technical Manual

Maxwell[®] 16 Polyhistidine Protein Purification Kit

INSTRUCTIONS FOR USE OF PRODUCT AS1060.



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Part# TM285

Maxwell[®] 16 Polyhistidine Protein Purification Kit

All technical literature is available on the Internet at: www.promega.com/tbs
Please visit the web site to verify that you are using the most current version of this Technical Manual. Please contact Promega Technical Services if you have questions on use of this system. E-mail: techserv@promega.com

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1. Description

The Maxwell[®] 16 Polyhistidine Protein Purification Kit^(a,b) is used with the Maxwell[®] 16 Instrument (Cat.# AS2000) to provide an easy method for efficient, automated purification of polyhistidine-tagged protein from bacterial cultures and other sample types, including mammalian cells, insect cells and culture medium. The kit also may be used for the purification of HQ-tagged proteins from bacterial cultures with some reagent modifications. The HQ tag contains three histidine and three glutamine residues (HQHQQH). The Maxwell[®] 16 Instrument is supplied with preprogrammed purification procedures and designed for use with predisposed reagent cartridges, maximizing simplicity and convenience. The instrument can process up to 16 samples in approximately 40 minutes, and the purified protein is compatible with common downstream applications, including polyacrylamide gel electrophoresis and Western blot analysis.

The Maxwell[®] 16 Polyhistidine Protein Purification Kit purifies samples using MagneHis[™] Ni-Particles^(a) to provide consistent protein purification of polyhistidine-tagged proteins. The MagneHis[™] Ni-Particles are paramagnetic precharged nickel particles that bind specifically to polyhistidine tags in recombinant proteins. The Maxwell[®] 16 Instrument is a magnetic-particle-handling instrument that efficiently transports the MagneHis[™] Ni-Particles

1. Description (continued)

through purification reagents in the prefilled cartridges (Figure 1) and mixes during processing. The paramagnetic-based methodology avoids common problems experienced with automated systems, such as clogged tips or partial reagent transfer, which can lead to suboptimal purification.

Processing Capacity

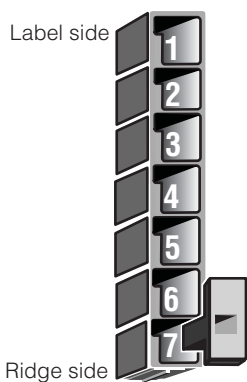
Table 1 describes the processing capacity of the Maxwell® 16 Polyhistidine Protein Purification Kit.

Table 1. Recommended Maximum Sample Amounts.

Sample Type	Processing Capacity
Bacterial Culture	Up to 20 O.D. ₆₀₀ ¹
Mammalian Cell Culture Cells	Up to 5 × 10 ⁶ cells ^{1,2}
Insect Cell Culture Cells	Up to 5 × 10 ⁶ cells ^{1,2}
Mammalian or Insect Cell Culture Medium	1ml ²

1. For ≥4 O.D.₆₀₀ bacterial cells or ≥2 × 10⁶ mammalian or insect cells, DNase I must be added for proper sample processing. All samples must be processed in a 1ml volume.
2. For mammalian cells and insect cells or culture medium containing serum, the addition of 200–500mM NaCl to the wells containing wash buffer can decrease nonspecific binding.

	Contents	User Adds:
Label side	1 Lysis Buffer	Sample
	2 MagneHis™ Ni-Particles	
	3 Wash Buffer	
	4 Wash Buffer	
	5 Wash Buffer	
	6 Wash Buffer	
Ridge side	7 Empty	Plunger



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Figure 1. Maxwell® 16 Polyhistidine Protein Purification Sample Cartridge.

2. Product Components and Storage Conditions

Product	Size	Cat.#
Maxwell® 16 Polyhistidine Protein Purification Kit	48 preps	AS1060

Sufficient for 48 automated isolations from bacterial samples. Includes:

- 48 Maxwell® 16 Polyhistidine Protein Purification Sample Cartridges
- 50 Plungers
- 50 Elution Tubes
- 16ml MagneHis™ Elution Buffer

Storage Conditions: Store the Maxwell® 16 Polyhistidine Protein Purification Kit at 2-10°C.

3. Maxwell® 16 Instrument Firmware Setup

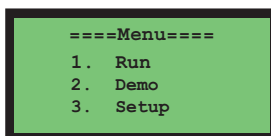
The first time the instrument is powered up, a series of user prompts will appear on the Navigation LCD. The Maxwell® 16 Polyhistidine Protein Purification Kit is intended to be used in the “Research” mode with the “Protein” method. If this method is not a choice on the Run Screen (Section 5, Step 10), please contact Technical Services for the latest firmware version.

Email: techserv@promega.com

Once the Research mode is set up on the instrument, all subsequent power-ups of the instrument will automatically default to these settings.

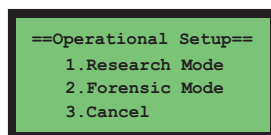
Before beginning your first purification using the Maxwell® 16 Instrument, it is necessary to set up the correct method on the instrument. Please refer to the following steps:

1. Turn on the instrument. At the Menu screen, use the Scroll button on the Navigation LCD to move the cursor to choice #3 “Setup”.



```
====Menu====  
1. Run  
2. Demo  
3. Setup
```

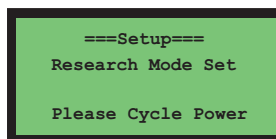
2. Press the “Run/Stop” button to select.
3. At the Setup screen, use the Scroll button on the Navigation LCD to move the cursor to choice #1 “Research Mode”.



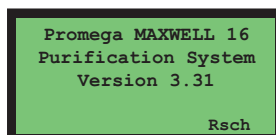
```
==Operational Setup==  
1. Research Mode  
2. Forensic Mode  
3. Cancel
```

3. Maxwell® 16 Instrument Firmware Setup (continued)

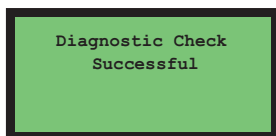
4. Press the “Run/Stop” button to select.
5. After pressing the “Run/Stop” button, turn the Maxwell® 16 Instrument off, wait a few seconds, then turn it back on to cycle the power.



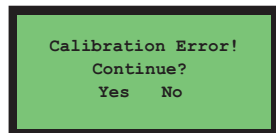
6. After turning the power on, you will briefly see a screen indicating the firmware version number and in the bottom right-hand corner the text “Rsch”, the abbreviation for Research Mode. Each time the instrument is turned on after this point, Research Mode will be the default setting.



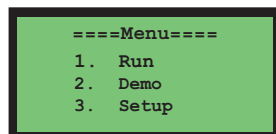
7. The instrument will automatically perform a diagnostic axis check to ensure the instrument is functioning properly. A screen will briefly appear indicating the test was successful.



If the diagnostic axis check was not successful, a “Calibration Error” screen will be shown. Refer to the *Maxwell® 16 Instrument Operating Technical Manual* #TM274 if this occurs.



8. If the diagnostic check was successful, the Menu screen will appear automatically following the diagnostic axis check. No additional setup is required. The instrument is now ready to purify protein samples using the Maxwell® 16 Polyhistidine Protein Purification Kit.



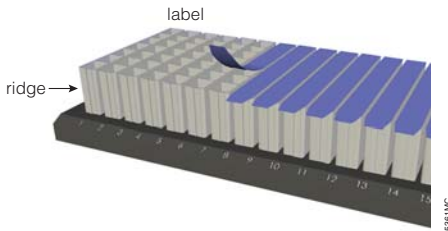
Note: The default settings can be changed to accommodate future laboratory needs. To change the default settings, refer to Step #1 above.

4. Sample Preprocessing

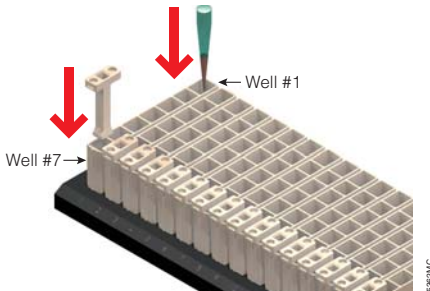
Materials to Be Supplied by the User

- 37°C incubator for flasks/tubes
- shaker
- appropriate culture medium
- bacterial cells, mammalian cells, insect cells, or culture medium samples
- 100mM HEPES (pH 7.5) if processing cell pellets
- DNase I (Sigma Cat.# D4527; resuspended to 5mg/ml in 10mM Tris-HCl [pH 7.5], 50mM NaCl, 10mM MgCl₂, 1mM DTT, and 50% glycerol), if processing ≥ 4 O.D.₆₀₀ bacterial cells per sample or $\geq 2 \times 10^6$ mammalian or insect cells per sample

4.A. Preprocessing Protocol by Sample Type



1. Place the number of cartridges to be used into the cartridge preparation rack. Place each cartridge into the holder with the ridged side of the cartridge facing towards the numbered side of the rack and the label side of the cartridge toward the back (Figure 1). Hold the cartridge firmly and remove the seal.



2. Place one plunger into well #7 of each cartridge. (Well #7 is the well closest to the ridged side of the cartridge and closest to the numbered front of the rack).
Note: The plunger will fit loosely in the cartridge.

4.A. Preprocessing Protocol by Sample Type (continued)

Bacterial Cultures Expressing a Polyhistidine-Tagged Protein

Bacterial cultures can be grown in tubes, flasks or 96-well plates. Grow the culture containing the appropriate polyhistidine-tagged protein to an O.D.₆₀₀ between 0.4 and 0.6, then induce protein expression. For IPTG induction, add IPTG to a final concentration of 1mM, and incubate at 37°C for 3 hours or at 25°C overnight. Absorbance measurements at 600nm should be performed on cultures diluted at least 1:10 to 1:50 in growth medium, and the spectrophotometer should be blanked with the same growth medium.

The following bacterial growth media are compatible with this system: Luria Broth (LB), Terrific Broth (TB), and CIRCLEGROW® media. Up to 20 O.D.₆₀₀ of culture may be processed per sample. However, for samples containing ≥ 4 O.D.₆₀₀, we recommend adding 10-50 μ g/ml DNase I prior to processing (i.e., 2-10 μ l of the 5mg/ml stock to a 1ml sample). This will decrease the viscosity of the subsequent lysate and increase processing efficiency and yield.

Samples must be processed as 1ml volumes, and the protein purification can be performed directly from culture or by first pelleting the cells and then resuspending the pellet in 1ml 100mM HEPES (pH 7.5). Some polyhistidine-tagged clones purify more efficiently from a cell pellet, so we recommend initially comparing purification directly from culture to purification from a cell pellet. In addition, some clones may purify more efficiently after a 3- 16-hour incubation at 4°C following growth and induction. This extra incubation step may aid in protein folding. This system has been used successfully with a number of different bacterial strains including BL21(DE3), BL21(DE3)pLysS and JM109(DE3).

Standard Protocol for Bacterial Cultures

For bacterial cultures in which the O.D.₆₀₀ is <4 , add 1ml of culture or cells resuspended in 1ml of 100mM HEPES, and add to well #1 of the cartridge. Proceed to Section 5, Step 1.

Note: Some strains of bacteria, such as those containing pLysS or pLysE, may require DNase even with <4 O.D.₆₀₀ of culture.

High-Density Bacterial Culture Protocol

For bacterial cultures in which the O.D.₆₀₀ is ≥ 4 or the total O.D. processed will exceed 4, add 1ml of culture, or cells resuspended in 1ml of 100mM HEPES, to well #1. Add 2-10 μ l of the 5mg/ml DNase I to well #1 and proceed to Section 5, Step 1. Up to 20 O.D.₆₀₀ can be processed per cartridge.

Mammalian Cells or Insect Cells Expressing a Polyhistidine-Tagged Protein

Mammalian cells or insect cells can be grown in tubes, flasks or 96-well plates. We recommend adding 200–500mM NaCl to the four wells of wash buffer (i.e., 40–100µl 5M NaCl per well) for mammalian or insect cell samples, particularly those in medium that contains serum. This additional salt in the wash buffer reduces the background often associated with serum and these cell types. Up to 5×10^6 mammalian or insect cells may be processed per sample, but with samples containing $\geq 2 \times 10^6$ cells, we recommend adding 10–50µg/ml DNase I to the sample prior to processing (i.e., 2–10µl of the 5mg/ml stock to a 1ml sample). This will decrease the viscosity of the subsequent lysate and increase processing efficiency and yield.

Samples must be processed as 1ml volumes, and the protein purification can be performed directly from culture or by first pelleting the cells and then resuspending the pellet in 1ml 100mM HEPES (pH 7.5). Some polyhistidine-tagged clones purify more efficiently from a cell pellet, so we recommend initially comparing purification directly from culture to purification from a cell pellet. Proceed to Section 5, Step 1.

Culture Medium Containing a Polyhistidine-Tagged Protein

Up to 1ml samples of culture medium may be processed per sample. We recommend adding 200–500mM NaCl to the four wells of wash buffer (i.e., 40–100µl 5M NaCl per well) for culture medium samples that contain serum. The salt in the wash buffer reduces the background often associated with serum. If no serum is present in the culture medium the Maxwell® 16 Polyhistidine Protein Purification Kit may be used without modifications. Proceed to Section 5, Step 1.

Bacterial Cultures Expressing an HQ-Tagged Protein

Bacterial cultures can be grown in tubes, flasks or 96-well plates. Grow the culture containing the appropriate HQ-tagged protein to an O.D.₆₀₀ between 0.4 and 0.6, then induce protein expression. For IPTG induction, add IPTG to a final concentration of 1mM, and incubate at 37°C for 3 hours or at 25°C overnight.

The following bacterial growth media are compatible with this system: Luria Broth (LB), Terrific Broth (TB), and CIRCLEGROW® media. Up to 20 O.D.₆₀₀ of culture may be processed per sample, but with samples containing ≥ 4 O.D.₆₀₀ we recommend adding 10–50µg/ml DNase I prior to processing (i.e., 2–10µl of the 5mg/ml stock to a 1ml sample). This will decrease the viscosity of the subsequent lysate and increase processing efficiency and yield.

Samples must be processed as 1ml volumes, and the protein purification can be performed directly from culture or by first pelleting the cells and then resuspending the cell pellet in 1ml 100mM HEPES (pH 7.5). We recommend adding 200–500mM NaCl to well #1 with the sample and the four wells of wash buffer (i.e., 40–100µl 5M NaCl per well) for bacterial cultures expressing HQ clones, as they purify more efficiently when NaCl is included in the lysis, binding and washing steps. We recommend initially comparing purification

directly from culture to purification from a cell pellet. In addition, some clones may purify more efficiently following a 3- 16-hour incubation at 4°C following growth and induction. This extra incubation step may aid in protein folding.

Follow the directions for the Standard Protocol or the High-Density Culture Protocol in Section 4.A and then Section 5, Step 1.

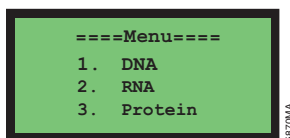
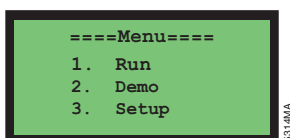
5. Maxwell® 16 Automated Protein Purification

1. Transfer your sample into well #1. (Well #1 is the well closest to the cartridge label and furthest from the user). The sample may be any of the following: 1ml bacterial culture or cell pellet resuspended in 1ml 100mM HEPES (pH 7.5); 1ml mammalian or insect cell culture or cell pellet resuspended in 1ml 100mM HEPES (pH 7.5); or 1ml culture medium.

Note: For bacterial samples ≥ 4 O.D.₆₀₀ or for $\geq 2 \times 10^6$ mammalian or insect cells, be sure to also add 10-50 μ g/ml DNase I to well #1.

Note: For mammalian or insect cells or culture, culture media with serum, or HQ-tagged clones you may need to add 200-500mM NaCl (40-100 μ l 5M NaCl) to the lysis and/or washing wells (wells #1 and 3-6). See Section 4.A. for details.

2. Turn the Maxwell® 16 Instrument on. The instrument will power up, display the firmware version number, proceed through a self-check and home all axes. Select “Run” to perform a purification.



3. Use one of the scroll buttons to move the cursor to “Protein” to perform a purification run. Press “Run/Stop” to select. The Maxwell® 16 Polyhistidine Protein Purification Kit is intended to be used with the “**Protein**” method. If this is not a choice on the Run Screen, please contact Technical Services for the latest firmware version. Email: techserv@promega.com
Verify that the sample type is “Protein” by selecting “OK”.



Warning. Pinch point hazard.

4. Open the door when prompted to do so on the LCD display. Press the “Run/Stop” button to extend the platform.



5. Transfer cartridges from the cartridge preparation rack onto the Maxwell® 16 platform. Ensure that the cartridges are placed into the Maxwell® 16 Instrument with the ridged side of the cartridge closest to the door.

Notes:

- The cartridges will fit into the instrument only in this orientation. If you have difficulty fitting the cartridge onto the platform, check that the cartridge is in the correct orientation.
- It is easiest to insert the cartridge by inserting the ridged side first and then pressing down on the back of the cartridge to “click” it into place.
- If you are processing fewer than 16 samples, center the reagent cartridges on the platform, spacing them evenly outwards from the center.



Do not start the instrument prior to performing Steps 6 and 7.

5. Maxwell® 16 Automated Protein Purification (continued)



6. Place a blue Elution Tube for each sample into the elution tube slots at the front of the platform.
7. Add 300µl of Elution Buffer to each Elution Tube. Verify plungers have been added to well #7.

Note: Ensure that the correct volume of Elution Buffer has been added to the Elution Tubes prior to starting the automated method.

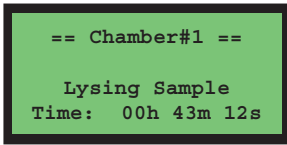
Note: For compatibility with downstream applications such as mass spectrometry analysis, alternative elution buffers may be used, such as 70% acetonitrile/0.1% TFA, or 0.1% TFA.

Note for HQ clones: HQ-tagged proteins may elute in lower concentrations of imidazole (as low as 50mM). The Elution Buffer included in the kit is at a concentration of 500mM imidazole. It may be diluted with water to a lower imidazole concentration and 300µl placed into the Elution Tubes. We recommend performing an imidazole titration to optimize elution of your HQ-tagged target protein.



Warning. Pinch point hazard.

8. Press the "Run/Stop" button. The platform will retract. Close the door.

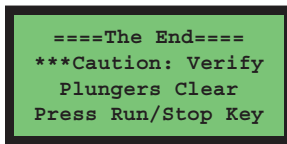


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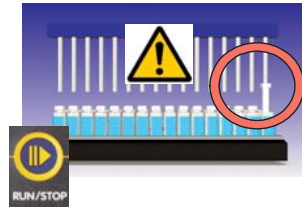
9. The Maxwell® 16 Instrument will begin the purification run immediately. The LCD screen will display the steps performed and the approximate time remaining in the run.

Notes:

- Pressing the “Run/Stop” button or opening the door will pause the run. Close the door (if open), and select whether to continue or terminate the run.
- If you select to “terminate” the program before completion, the instrument will wash the particles off the plungers and eject the plungers into well #1 of the cartridge, and **your sample will be lost**.

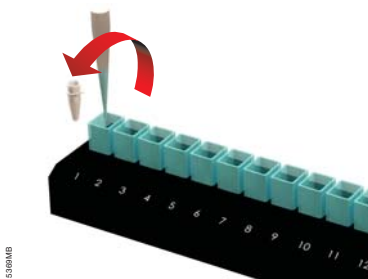


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10. When purification is complete, the LCD screen will display a message that the method has ended. Upon method completion, open the instrument door. Check to make sure that all of the plungers have been removed from the magnetic rod assembly. If the plungers have not been removed, push them down gently by hand to remove them from the magnetic rod assembly.
11. Press the “Run/Stop” button to extend the platform.

5. Maxwell® 16 Automated Protein Purification (continued)



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- Remove the Elution Tubes from the elution tube slots, and place them into the Magnetic Elution Tube Rack. Any residual particles will be captured along the back of the blue Elution Tubes. Transfer the eluted samples into storage tubes by pipetting away from the captured particles.

Note: Aspirate samples from the elution tubes by placing the pipette tip on the front side of the elution tube. A small volume of liquid may remain in the elution tube.

- Remove cartridges and plungers from the instrument platform and discard. **Do not** reuse Reagent Cartridges, Plungers or Elution Tubes.

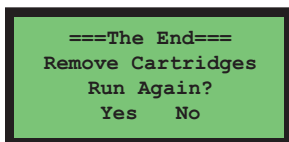


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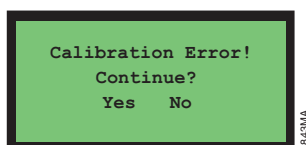
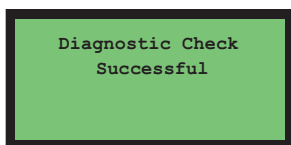
- Use one of the scroll buttons to move the cursor to select "Yes" or "No" to run the purification method again.

If "Yes" is selected, the Menu screen will appear (see Section 3, Step 8).

If "No" is selected, the platform is retracted back into the instrument. You are then prompted to close the door.



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15. A diagnostic axis check is automatically performed whether another run is chosen or not. If the check is successful, the LCD screen will display a message indicating so. If the check is unsuccessful, an error message will appear. Refer to the *Maxwell® 16 Instrument Operating Technical Manual #TM274* for further information about resolving the error.

6. General Considerations

1. Purified polyhistidine- or HQ-tagged protein can be quantitated using standard methods such as Bradford or BCA, but the imidazole in the Elution Buffer may interfere with these assays. Either dialyze the sample or dilute to the optimal imidazole concentration for the protein quantitation reagent. The Pierce BCA assay (Pierce Cat.# 23225) may be used with undiluted samples with the macroscale assay. Always include the same amount of imidazole in the standard curve for optimal accuracy.
2. Polyhistidine proteins expressed for purification with this system should have at least five to six consecutive histidine residues located on the N- or C-terminus. The HQ tag contains three histidine and three glutamine residues (HQHQHQ).
3. The Binding/Wash Buffer contains 10mM imidazole to prevent nonspecific binding to the MagneHis™ Ni-Particles.
4. We have used the Maxwell® 16 Polyhistidine Protein Purification Kit to purify polyhistidine- or HQ-tagged proteins generated in vitro by *E. coli* S30 extract or wheat germ extract (TnT® SP6 High-Yield Protein Expression System, Cat.# L3260). We do not recommend the Maxwell® 16 Polyhistidine Protein Purification Kit for the purification of polyhistidine- or HQ-tagged proteins expressed in rabbit reticulocyte lysate-based in vitro translation systems due to the ability of hemoglobin to bind to the MagneHis™ Ni-Particles. For rabbit reticulocyte lysate-expressed proteins, we recommend the MagZ™ Protein Purification System (Cat.#V8830). For additional details, contact Promega Technical Services.

7. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

Symptoms	Causes and Comments
Low protein recovery or yield	<p>Low expression. Optimize expression by varying culture medium (LB, TB, or CIRCLEGROW® media), induction temperature, induction time, and amount of inducing agent. You may increase the volume of culture processed per sample by centrifuging the sample (10 minutes at 8,000rpm) and resuspending the pellet in 1ml 100mM HEPES (pH 7.5). No more than 20 absorbance units at 600nm can be processed per sample, and ≥ 4 O.D.₆₀₀ units require adding DNase I to reduce viscosity. No more than $\sim 5 \times 10^6$ mammalian or insect cells can be processed per sample. If $\geq 2 \times 10^6$ cells are used, adding DNase I is required.</p> <hr/> <p>Clone does not contain an intact polyhistidine or HQ tag. Sequence the recombinant protein clone to verify that it contains an intact polyhistidine or HQ tag. Moving the tag to the alternative end of the target protein may also enhance expression and/or purification. In our experience, N-terminal tags purify more efficiently than C-terminal tags.</p> <hr/> <p>Poor processing. Samples must be processed in 1ml aliquots for optimal lysis buffer concentration. Verify that you have not exceeded the maximal number of cells that can be processed. Be sure to add DNase I. We recommend diluting a bacterial culture at least 1:10 to 1:50 in the appropriate culture medium prior to absorbance measurements to obtain an accurate O.D.₆₀₀ reading. Be sure to blank the spectrophotometer with medium alone.</p> <hr/> <p>Poor elution. If 500mM imidazole is not sufficient for elution of the target polyhistidine-tagged protein, place 300µl of an alternative lysis buffer into the blue Elution Tube. Elution may be performed in 1M imidazole, 1X SDS Laemmli sample buffer, TFA, an acetonitrile/trifluoroacetic acid mixture, or other.</p>

Symptoms	Causes and Comments
Low protein recovery or yield (continued)	<p>Expressed protein is unstable. If the expressed protein is unstable, protease inhibitors may be added during lysis. Add the protease inhibitors to the cell culture or resuspended cell pellet and then proceed to Section 5, Step 3.</p> <p>The expressed protein may be expressed as inclusion bodies, and protein purification might require denaturing conditions, such as resuspending the cell pellet in 2-8M guanidine-HCl or urea prior to proceeding to Section 5, Step 2. This procedure may not be successful for all inclusion body-expressed proteins.</p>

8. Related Products

Product	Size	Cat.#
Maxwell® 16 Instrument*	1 each	AS2000
Maxwell® 16 Blood DNA Purification Kit*	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit*	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit*	48 preps	AS1030
Maxwell® 16 Total RNA Purification Kit*	48 preps	AS1050
Maxwell® 16 Tissue LEV Total RNA Purification Kit*	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit*	48 preps	AS1225
DNA IQ™ Casework Kit for Maxwell® 16 **	48 preps	AS1210
DNA IQ™ Reference Sample Kit for Maxwell® 16**	1 kit	AS1040

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

^(a)Patent Pending.

^(b)This product is licensed for use under U.S. Pat. No. 6,174,704.

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TNT is a registered trademark of Promega Corporation. DNA IQ, MagneHis, MagZ and Maxwell are trademarks of Promega Corporation.

CIRCLEGROW is a registered trademark of Qbiogene, Inc.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.