Promega

Technical Bulletin

MagZ™ Protein Purification System

INSTRUCTIONS FOR USE OF PRODUCT V8830.

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MagZ™ Protein Purification System

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 Bulletin. Please contact Promega Technical Services if you have questions on use of this system. E-mail techserv@promega.com.

1. Description

Purification of a polyhistidine- or HQ-tagged protein that has been expressed in rabbit reticulocyte lysate is currently limited by hemoglobin in the lysate copurifying with the protein of interest. Hemoglobin copurification limits downstream applications (e.g., fluorescence-based functional assays, protein-protein interaction studies) and reduces the amount of protein purified. Thus, we developed a unique system that purifies polyhistidine- or HQ-tagged proteins expressed in rabbit reticulocyte lysate. These polyhistidine- or HQ-tagged proteins are 99% free of the contaminating hemoglobin.

The MagZ™ Protein Purification System[a] provides a simple, rapid and reliable method for the purification of expressed polyhistidine- or HQ-tagged protein from rabbit reticulocyte lysate. Paramagnetic precharged particles can be used to isolate polyhistidine- and HQ-tagged protein from 50–500µl of TNT® Reticulocyte Lysate[b–e] with minimal copurification of hemoglobin.

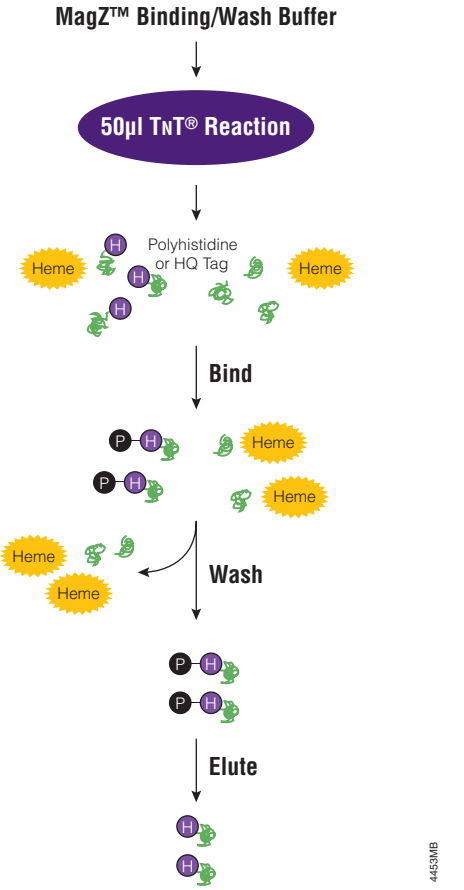

Figure 1. Schematic of the MagZ™ Protein Purification System. Lysate containing the polyhistidine- or HQ-tagged protein is diluted with MagZ™ Binding/Wash Buffer and MagZ™ Binding Particles are added. Polyhistidine- or HQ-tagged proteins bind to the MagZ™ Binding Particles within 15 minutes. Unbound proteins are washed away, and the target protein is recovered by elution with MagZ™ Elution Buffer.

The MagZ™ Protein Purification system has the following features:

Specific: Minimal hemoglobin (<0.1%) binding to the MagZ™ Binding Particles.

Flexible: MagZ™ Binding Particles are compatible with a variety of common buffers.

Quick: No long incubations are required.

Scalable: Volumes can be adjusted from 50–500µl.

Convenient: Complete system that includes all necessary components for purification.

Versatile: Binding, wash and elution conditions can be further optimized for individual polyhistidine- or HQ-tagged proteins with easy combinations of the MagZ™ Binding/Wash and Elution Buffers.

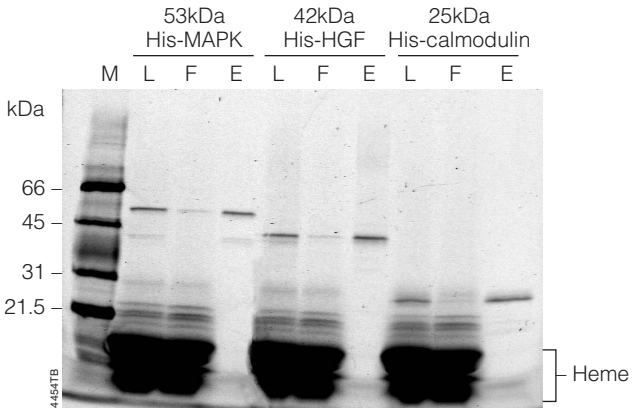

Figure 2. Purification of Polyhistidine-tagged proteins using the MagZ™ Protein Purification System. Proteins were expressed using the TNT® T7 Quick Coupled Transcription/Translation System[b–f] (Cat.# L1170), 1µg of DNA and 2µl of FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System (Cat.# L5001). The reactions were incubated at 30°C for 60 minutes. The polyhistidine-tagged protein was purified using the MagZ™ Protein Purification System and analyzed by SDS-PAGE. The FluoroTect™-Labeled proteins were visualized using a FluorImager® instrument with a 530nm filter. Lanes L, 2µl lysate; lanes F, 6µl flowthrough; lanes E, 8µl eluate.

2. Product Components and Storage Conditions

Product	Size	Cat.#
MagZ™ Protein Purification System	30 purifications	V8830

Each system contains sufficient reagents for purification from thirty 50µl TnT® reactions. Includes:

- 2ml MagZ™ Binding Particles
- 40ml MagZ™ Binding/Wash Buffer
- 20ml MagZ™ Elution Buffer

Storage Conditions: Store all system components at 4°C. **Do not freeze the MagZ™ Binding Particles.**

3. MagZ™ Protein Purification Protocol

Materials to be Supplied by the User

- Platform shaker, platform rocker, rotary platform or rotator
- MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5331, Z5332, Z5341 or Z5342)
  Note: Promega supplies magnetic separation devices that can be used to hold 1–12 tubes.

3.A. General Considerations

1. A TnT® reaction typically produces 100–300ng of protein per 50µl of reaction volume.
2. Expression levels of polyhistidine- or HQ-tagged proteins are protein-dependent and will vary.
3. Polyhistidine-tagged proteins expressed for purification with this system should have at least five consecutive histidine residues located on the C- or N-terminus.
4. The MagZ™ Protein Purification System is optimized to reduce hemoglobin binding while maximizing the recovery of the expressed fusion proteins.
5. ^{35}S- or FluoroTect™-labeled polyhistidine- and HQ-tagged proteins can be purified.
6. Binding/wash and elution conditions can be optimized (see Section 3.B)
7. MagZ™ Binding Particles have been evaluated for compatibility with several common buffer components (Table 1).
8. The MagZ™ Binding Particles should be stored and handled carefully to avoid contamination. Always use new pipette tips.

3.B. Protocol

TNT® Reaction/ MagZ™Binding/Wash Buffer Solution

Prepare a TNT® reaction/MagZ™ Binding Buffer solution following protein synthesis using the TNT® Coupled Reticulocyte Lysate, TNT® Quick Coupled Transcription/Translation (Cat.# L1170, L2080) or TNT® T7 Quick for PCR DNA Systems[c,d,f] (Cat.# L5540).

Note: For more information about Protein Synthesis with these TNT® Systems, please see the *TNT® Coupled Reticulocyte Lysate System Technical Bulletin* #TB126, the *TNT® Quick Coupled Transcription/Translation Systems Technical Manual* #TM045 or the *TNT® T7 Quick for PCR DNA Technical Manual* #TM235.

1. Add 100µl of MagZ™ Binding/Wash Buffer to 50µl of the TNT® reaction.
2. Mix by inverting or pipetting slowly 5 times. To prevent foaming, do not mix vigorously.

Particle Preparation

3. Mix the MagZ™ Binding Particles to a uniform suspension using a vortex mixer.
4. Transfer a 60µl aliquot of particles to a 0.5 or 1.5ml tube.
5. Place the tube in the appropriate magnetic stand for approximately 15 seconds. Allow the MagZ™ Binding Particles to be captured by the magnet. Carefully remove the supernatant, and proceed immediately to Step 6.

   Note: If the captured particles are at the bottom of the tube and you have difficulty removing the liquid without particle contamination, resuspend the particles by pipetting without removing the tube from the magnetic stand. This will allow the particles to be captured higher up on the side of the tube.

Protein Binding

6. Add 150µl of TNT® reaction/MagZ™ Binding/Wash Buffer mix to the prepared particles.
7. Carefully pipette the mixture to resuspend the particles. Avoid foaming.
8. Incubate tubes for 15 minutes with mixing. The tube can be flicked, mixed by pipetting periodically or placed on a shaker or rocking platform to keep the particles from settling.

Do not allow the particles to settle during incubation, as this will decrease the binding efficiency.

9. Place the tube on the appropriate magnetic stand for approximately 15 seconds. Allow the MagZ™ Binding Particles to be captured by the magnet. Carefully remove the supernatant. Collection of this supernatant for analysis is optional.

Washing

10. Remove the tube from the magnet. Add 200µl of MagZ™ Binding/Wash Buffer to the MagZ™ Particles and mix by pipetting. Make sure that the particles are fully resuspended.

    Note: Carefully rinse the cap and sides of the tube to wash off any particles or TnT® reaction/MagZ™ Binding/Wash Buffer.

11. Place the tube in the appropriate magnetic stand for approximately 15 seconds. Allow the MagZ™ Particles to be captured by the magnet, and carefully remove the supernatant.

12. Repeat steps 10 and 11 three times for a total of four washes.

Elution

13. Add 100µl of MagZ™ Elution Buffer to the tube and mix by pipetting 5 times.

    Note: Elution with 1M imidazole will produce the highest yield of polyhistidine- or HQ-tagged protein; however, nonspecifically bound proteins will also elute off at this concentration. If you wish to optimize your elution conditions, see "Optional Optimization of Elution Conditions".

14. Immediately place the tube in the appropriate magnetic stand for approximately 15 seconds. Allow the MagZ™ Particles to be captured by the magnet.

15. Keeping the tube in the magnetic stand, carefully remove the supernatant containing the purified protein. Wait an additional 10 seconds and collect any additional liquid. If the eluted sample contains some MagZ™ Particles, transfer the eluate to a new tube, place eluate tube in the magnetic stand and collect the eluate to a new tube.

Optional Optimization of Elution Conditions

Many polyhistidine- or HQ-tagged proteins elute off the MagZ™ Binding Particles using less than 1M imidazole. If you wish to optimize the elution condition, the concentration of the MagZ™ Elution Buffer can be titrated by diluting the buffer with Nuclease-Free Water. Suitable imidazole concentration for elution can be determined by increasing the concentration of imidazole sequentially from lowest to highest concentration, such as 50–1000mM. Collect each flowthrough for analysis. Choose the elution conditions that best meets your needs. For alternative elution conditions, see Section 3.D.

Note: HQ-tagged proteins may elute with a lower concentration of imidazole (50–100mM) compared to polyhistidine-tagged proteins.

3.C. Gel Analysis

^{35}S-Labeled Protein

1. Combine 1–5µl of the eluate from the [^{35}S]methionine-labeled sample and 5µl of 4X SDS gel-loading buffer; add Nuclease-Free Water to a final volume of 20µl.
2. Incubate samples for 5 minutes at 95°C.

   Note: In some cases, high molecular weight complexes are formed at 100°C, and denaturation may need to be performed at lower temperatures (e.g., 20 minutes at 60°C or 3–4 minutes at 80–85°C).
3. Load the prepared samples onto a 4–20% Tris-glycine gel. The volume loaded will depend upon the well capacity. Run at 25–50mA until the dye front is close to the bottom of the gel.
4. Transfer the proteins to a PVDF membrane or dry the gel.
5. Visualize the proteins by exposing to film or using a PhosphorImager® instrument following the manufacturer's instructions.

FluoroTect™-Labeled Proteins

1. Combine 5–15µl of eluate from the FluoroTect™ Green$_{Lys}$ tRNA-labeled sample with 5µl of 4X SDS gel-loading buffer; add Nuclease-Free Water to a final volume of 20µl.
2. **Do not** heat FluoroTect™-labeled samples, as the fluorescent label will dissociate from the protein.
3. Load the prepared samples onto a 4–20% Tris-glycine gel. The volume loaded will depend upon the well capacity. Run at 25–50mAmp until the dye front is close to the bottom of the gel.
4. Visualize the gel using a FluorImager® instrument following the manufacturer's instructions.

3.D. Alternative Buffer Conditions

The MagZ™ Binding Particles have been evaluated for compatibility with several common buffer components (Table 1).

Table 1. Tolerance of MagZ™ Binding Particles Using Alternative Buffers.

Reagent	Maximum Concentration
Tris	100mM
HEPES	100mM
Triton® X-100	1%
Tween® 20	0.05%
NaCl	<1M
Glycerol	<20%
β-mercaptoethanol	<1mM

Alternative Binding Conditions

Optimal binding of polyhistidine- or HQ-tagged protein to the MagZ™ Binding Particles should take place in the absence of imidazole. Low concentrations of imidazole (up to 20mM) can be added in the MagZ™ Binding/Wash Buffer to reduce nonspecific binding (we have seen less than 1% background), but the amount of polyhistidine- or HQ-tagged protein purified may be decreased. Binding of polyhistidine- or HQ-tagged proteins to MagZ™ Binding particles in the presence of imidazole is protein-dependent.

Alternative Elution Conditions

Many polyhistidine- or HQ-tagged proteins elute off the MagZ™ Particles with <1M imidazole. See Section 3.B, "Optional Optimization of Elution Conditions" for information about titration of the imidazole concentration. Table 2 shows examples of polyhistidine-tagged proteins that elute at different imidazole concentrations. For certain applications, alternative elution conditions may be required. Elution conditions may be optimized or altered as needed (Table 3).

Table 2. Example of Polyhistidine-tagged Proteins that Elute at Different Imidazole Concentrations.

Polyhistidine-tagged Protein	Imidazole Concentration
Polyhistidine-actin	1M
Polyhistidine-VEGH	1M
Polyhistidine-calmodulin	50–100mM
Polyhistidine-MAPK	50–100mM
Polyhistidine-activin	500mM
Polyhistidine-JNK	500mM
Polyhistidine-Akt	500mM

Table 3. Alternative Elution Conditions.

Condition	Effect
Decrease volume of MagZ™ Elution Buffer	Concentrates the protein.
100mM histidine	Eliminates imidazole inhibition.
Decrease elution to pH 6.5	May improve amount of polyhistidine- or HQ-tagged protein recovered.
Decrease imidazole concentration	Reduces the inhibitory effect of imidazole in downstream applications.
	Reduces background.

Note: Elution of polyhistidine- or HQ-tagged proteins is protein-specific.

3.E. Reaction Scale Up and Protein Concentration

Scale Up Reactions

Increase all reagents in proportion to the amount of TnT® reaction that will be used. For example, a 500µl TnT® reaction would be diluted with 1ml of MagZ™ Binding/Wash Buffer and added to 600µl of MagZ™ Binding Particles. The particles would be washed 4 times with 2ml of MagZ™ Binding/Wash Buffer and the polyhistidine- or HQ-tagged protein eluted with two additions of 500µl of MagZ™ Elution Buffer. To concentrate the protein, elution may be performed with a smaller volume.

Concentrating Polyhistidine- or HQ-Tagged Proteins on the MagZ™ Binding Particles

The amount of polyhistidine- or HQ-tagged protein bound to the MagZ™ Binding Particles can be increased by adding multiple 50µl TnT® reactions to the MagZ™ Particles and allowing the polyhistidine- or HQ-tagged protein to bind. To do this, repeat the binding step several times before proceeding to the elution step. Place in the magnetic stand and remove the supernatant. The polyhistidine- or HQ-tagged protein is eluted with 50µl of MagZ™ Elution Buffer.

4. 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
No protein purified	No expression. Please refer to the appropriate TNT® System Technical Literature for Troubleshooting of protein expression.
	Check for polyhistidine protein by Western blotting using an anti-polyhistidine antibody. Check for HQ-tagged protein using an activity assay.
Problem with downstream applications	Inhibition by imidazole. Elute using one of the alternative methods (Table 3).
	Decrease imidazole concentration in elution.
	Use a Microcon® filter to remove imidazole.
Protein not binding or low yield	Protein is is missing purification tag or is degraded. Check polyhistidine tag by Western blotting using an anti-polyhistidine antibody. Check for HQ-tagged protein using an activity assay.
	Purification tag not accessible. Clone into a vector with tag in opposite location (e.g., N-terminal versus C-terminal.)
	Polyhistidine- or HQ-tagged protein not eluting. Try one of the alternative elution conditions (Table 3.)
	Remove eluted polyhistidine- or HQ-tagged protein from the MagZ™ Binding Particles immediately. Prolonged incubations may reduce the amount of polyhistidine- or HQ-tagged protein recovered.

5. Composition of Buffers and Solutions

MagZ™ Binding/Wash Buffer (pH 7.4)

20mM sodium phosphate
500mM NaCl

MagZ™ Elution Buffer

1M imidazole (pH 7.5)

4X SDS gel-loading buffer

0.24M Tris-HCl (pH 6.8)
2% SDS
3mM bromophenol blue
50.4% glycerol
0.4M dithiothreitol

SDS gel-loading buffer lacking dithiothreitol can be stored at room temperature. Dithiothreitol should be added from a 1M stock just before the buffer is used.

6. Related Products

Product	Size	Cat.#
TNT® T7 Quick for PCR DNA*	40 reactions	L5540
TNT® T7 Quick Coupled Transcription/Translation System*	40 reactions	L1170
TNT® SP6 Quick Coupled Transcription/Translation System*	40 reactions	L2080
FluoroTect™ $Green_{Lys}$ in vitro Translation Labeling System*	40 reactions	L5001
MagneSphere® Technology Magnetic Separation Stand (two-position)	0.5ml	Z5331
	1.5ml	Z5332
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
	1.5ml	Z5342

* For Laboratory Use.

HQ Tag Flexi® Vectors

Product	Size	Cat.#
pFN6A (HQ) Flexi® Vector	20µg	C8511
pFN6K (HQ) Flexi® Vector	20µg	C8521
pFC7A (HQ) Flexi® Vector	20µg	C8531
pFC7K (HQ) Flexi® Vector	20µg	C8541

pFN6A and pFN6K (HQ) Flexi® Vectors: These vectors are designed for expressing N-terminal, HisGln (HQ) metal-binding peptide fusion proteins in bacteria and in vitro protein expression systems. The vectors are configured to append the peptide sequence MKHQHQHQAIA to the amino terminus of a protein. The vectors

are designed for bacterial or in vitro protein expression via the T7 RNA polymerase promoter and are available with ampicillin (pFN6A (HQ) Flexi® Vector) or kanamycin (pFN6K (HQ) Flexi® Vector) resistance for selection in *E. coli*.

pFC7A and pFC7K (HQ) Flexi® Vectors: These vectors are designed for expressing C-terminal, HisGln (HQ) metal-binding peptide fusion proteins in bacteria and in vitro protein expression systems. The vectors are configured to append the peptide sequence VSHQHQHQ to the carboxy terminus of a protein. The vectors are designed for bacterial or in vitro protein expression via the T7 RNA polymerase promoter and are available with ampicillin (pFC7A (HQ) Flexi® Vector) or kanamycin (pFC7K (HQ) Flexi® Vector) resistance for selection in *E. coli*.

For further information regarding these HQ Flexi® Vectors and the Flexi® Vector Systems for cloning, refer to the *Flexi® Vector System Technical Manual* #TM254 or visit: **www.promega.com**

[a]Patent Pending.

[b]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.

[c]U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687, Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

[d]U.S. Pat. Nos. 5,324,637 and 5,492,817, European Pat. No. 0 566 714 B1, Australian Pat. No. 660329 and Japanese Pat. No. 2904583 have been issued to Promega Corporation for coupled transcription/translation systems that use RNA polymerases and eukaryotic lysates.

[e]U.S. Pat. Nos. 5,283,179, 5,641,641, 5,650,289 and 5,814,471, Australian Pat. No. 649289, European Pat. No. 0 553 234 and Japanese Pat. No. 3171595 have been issued to Promega Corporation for a beetle luciferase assay method, which affords greater light output with improved kinetics as compared to the conventional assay. Other patents are pending.

[f]U.S. Pat. No. 5,552,302, European Pat. No. 0 422 217, Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.