TECHNICAL MANUAL

MagneGST™ Pull-Down System

Instructions for Use of Product
V8870

Revised 5/17
TM249

MagneGST™ Pull-Down System

All technical literature is available at: www.promega.com/protocols/
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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

Many proteins function in association with partner proteins or as components of large multiprotein complexes. Understanding these protein interactions is critical to our understanding of biological pathways and cellular function. The yeast two-hybrid system is commonly used to determine protein:protein interactions but generates many false positives, requiring confirmation of results by another method. GST pull-down (1) is becoming an important tool for validation of suspected protein:protein interactions or for discovering novel protein interactions. GST pull-down uses a GST-fusion protein (bait) bound to glutathione (GSH)-coupled particles to affinity purify any proteins (prey) that interact with the bait from a pool of proteins in solution (2). Bait and prey proteins can be obtained from multiple sources including cell lysates, purified proteins, expression systems and in vitro transcription/translation systems.

1. Description (continued)

The MagneGST™ Pull-Down System[a–c] provides GSH-linked magnetic particles that allow simple immobilization of GST-fusion bait proteins from bacterial lysates and an in vitro transcription/translation system for expression of prey proteins. Prey proteins are isolated using the appropriate bait protein attached to the MagneGST™ (GSH) Particles. The MagneGST™ Pull-Down System protocol can be used for detection of interactions between GST-fusion proteins expressed in bacterial lysates and prey proteins expressed in the TnT® System. The protocol is divided into three phases: 1) the prey protein is expressed in the transcription/translation reaction; 2) the *E. coli*-expressed GST-fusion (bait) protein is immobilized onto the MagneGST™ Particles; and 3) the prey protein is added to the MagneGST™ Particles carrying bait and captured through bait-prey interaction. Phases 1 and 2 are performed simultaneously (Figure 1).

The transcription/translation component of the MagneGST™ Pull-Down System is the TnT® T7 Quick Master Mix, which allows the convenient single-tube coupled transcription/translation of genes cloned downstream from T7 RNA polymerase promoters. The TnT® System is compatible with circular (plasmid) or linear (plasmid or PCR product) templates. For immobilization and purification of bait proteins, the MagneGST™ Pull-Down System provides the MagneGST™ Cell Lysis Reagent to lyse bacterial cells and MagneGST™ Particles for binding GST-fusion bait proteins. Prey protein synthesized in the TnT® T7 Quick Coupled Transcription/Translation System reaction is captured using bait protein (GST-fusion protein) immobilized on MagneGST™ Particles. Nonspecifically bound proteins are then washed away and the prey protein is analyzed. Prey proteins can be detected by incorporating radioactively labeled methionine in the TnT® T7 Quick reaction, followed by SDS-PAGE and autoradiography, or by incorporating the supplied non-radioactive methionine in the TnT® reaction and detecting by Western blotting with specific antibodies.

Note: For more information on the TnT® T7 Quick Coupled Transcription/Translation System or MagneGST™ Protein Purification System, please refer to the *TnT® T7 Quick Coupled Transcription/ Translation System Technical Manual* #TM045 or the *MagneGST™ Protein Purification System Technical Manual* #TM240. All Promega Technical Manuals are available at: **www.promega.com/protocols/**

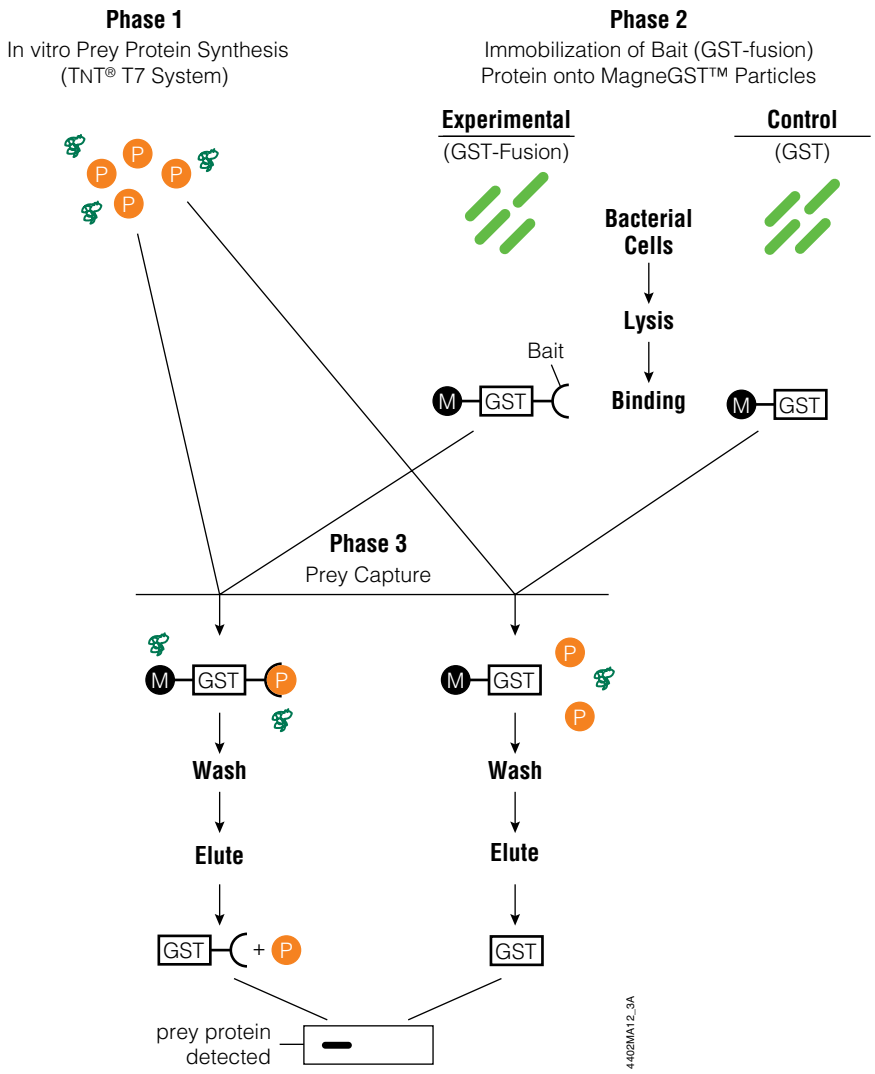

Figure 1. Overview of the MagneGST™ Pull-Down System protocol.
P = Prey Protein, M = MagneGST™ Particle.

2. Product Components and Storage Conditions

PRODUCT	CAT.#
MagneGST™ Pull-Down System	**V8870**

Each system consists of two individual parts, each with different storage conditions. Sufficient reagents to perform approximately 80 pull-down reactions are provided. Includes:

V8871 (Part 1 of 2 for V8870)
- 8 × 200µl TnT® T7 Quick Master Mix
- 50µl Methionine
- 1.25ml Nuclease-Free Water
- 1,000 units RQ1 RNase-Free DNase

V8872 (Part 2 of 2 for V8870)
- 4ml MagneGST™ Glutathione Particles
- 60ml MagneGST™ Cell Lysis Reagent
- 2 × 200ml MagneGST™ Binding/Wash Buffer

Storage Conditions: The expiration date for the TnT® T7 Quick Master Mix is listed on the product vial. Store the TnT® T7 Quick Master Mix and Methionine at –70°C. After first use, store the RQ1 RNase-Free DNase at –20°C. Nuclease-Free Water may be stored at –70°C, 4°C, or room temperature. Store the MagneGST™ Glutathione Particles, MagneGST™ Cell Lysis Reagent and the MagneGST™ Binding/Wash Buffer at 4°C. The MagneGST™ Cell Lysis Reagent may also be stored at room temperature.

The TnT® Quick Master Mix is shipped in foil packaging due to sensitivity of the system to carbon dioxide released from dry ice. Prolonged exposure to dry ice causes significant loss of activity.

Do not store the unfoiled lysate in the presence of dry ice. **Do not** freeze-thaw the TnT® T7 Quick Master Mix more than 2 times. **Do not** freeze the MagneGST™ Glutathione Particles.

Available Separately

The MagneGST™ Pull-Down System protocol requires a magnetic separation stand, which must be purchased separately.

PRODUCT	SIZE	CAT.#
MagneSphere® Technology	**1.5ml**	**Z5342**
Magnetic Separation Stand (12-position)	**12 × 75mm**	**Z5343**
MagneSphere® Technology	**1.5ml**	**Z5332**
Magnetic Separation Stand (2-position)	**12 × 75mm**	**Z5333**
MagneSil® Magnetic Separation Unit*	**1 each**	**A2231**

*Accommodates a single 15ml or 50ml conical tube.

3. General Considerations

General recommendations for use of the MagneGST™ Pull-Down System are provided in this section. Please refer to Section 4 for specific protocol details.

3.A. Controls

Appropriate controls should be performed during each phase of the MagneGST™ Pull-Down assay.

In Phase 1, it is important to verify that the prey protein is expressed in the TnT® reaction. Load 1–5% of the TnT® reaction product onto an SDS-PAGE gel and verify the presence of the prey protein by autoradiography or by Western blotting with specific antibody.

In Phase 2, controls should be performed to show that the GST-fusion protein is present and that it is bound to the MagneGST™ Particles. To check the efficiency of immobilization of the GST-fusion protein (or GST alone), remove 3–5µl of the MagneGST™ Particles after immobilization of bait protein (or GST) onto the particles and add 1X SDS loading buffer to a total volume of 20µl. Boil for 5 minutes to elute the protein, then analyze by SDS-PAGE. The MagneGST™ Particles can be loaded onto the gel along with the loading buffer. The amount of GST-fusion protein immobilized onto the particles can be estimated by comparison of the intensity of the GST-fusion protein band to that of bands containing known amounts of the same protein.

In Phase 3, negative controls using lysate from *E. coli* overexpressing GST protein should be performed to eliminate false positives resulting from the nonspecific interaction of prey protein with the magnetic particles or GST.

3.B. Phase 1: Synthesis of Prey Protein

DNA used for the transcription/translation reaction should be pure and residual ethanol should be removed before the DNA is added to the TnT® T7 Quick Master Mix. DNA purified using the Wizard® *Plus* (Cat.# A7100) or Wizard® *Plus* SV Minipreps DNA Purification Systems (Cat.# A1330) or by standard alkaline lysis (3) is sufficiently clean for use in the TnT® T7 Quick coupled transcription/ translation reaction. For most plasmid constructs, optimal results are obtained using 1µg of plasmid DNA template in the TnT® reaction. However, we have used 0.2–2.0µg DNA and obtained satisfactory levels of translation. The use of more than 1µg plasmid does not necessarily increase the amount of protein produced. Linearized templates produced by restriction enzyme digestion of plasmids should be purified before use in the transcription/translation reaction. PCR-generated DNA templates can also be used.

Note: For more information on preparation of DNA templates, please refer to the *TnT® T7 Quick Coupled Transcription/Translation System Technical Manual* #TM045. All Promega Technical Manuals are available at: **www.promega.com/protocols/**

Buffer Composition

Avoid adding Ca^{2+} to the translation reaction. Ca^{2+} may reactivate the micrococcal nuclease used to destroy endogenous mRNA in the lysate and result in degradation of the DNA or mRNA template.

Leaky scanning for translation initiation can result in translation initiating at internal methionines. Optimizing the Mg^{2+} or K^{+} concentration may increase fidelity (4).

3.C. Phase 2: Immobilization of Bait Protein

Numerous protocols for cloning and overexpression of soluble GST-fusion proteins are available in many molecular biology handbooks (5). Solubility of the GST-fusion protein is very important for successful GST pull-down and should be verified before attempting the pull-down experiment. To evaluate solubility, centrifuge a small volume of lysed cells expressing the fusion protein at 16,000 × *g* for 40 minutes. Analyze both the pellet and supernatant for the presence of the GST-fusion protein by SDS-PAGE. If the protein is found in the pellet, it is possible that either the cells were not efficiently lysed or that the protein is expressed in inclusion bodies. Cell lysis may be enhanced by freeze-thawing the cell pellets prior to the addition of MagneGST™ Cell Lysis Reagent or by adding 1/10 volume of 10mg/ml lysozyme in 25mM Tris-HCl (pH 8.0). Increasing the volume of MagneGST™ Cell Lysis Reagent used may also increase lysis efficiency. Alternatively, sonication may be used to lyse the cells. Different bacterial strains will lyse with different efficiencies; for example, *E. coli* BL21 is more resistant to lysis than other strains.

Optimizing growth and induction conditions may increase solubility from inclusion bodies. Lowering induction temperature, altering the concentration of inducing agent, inducing for a shorter time at higher cell density and increasing aeration during growth are all options for either enhancing bait protein solubility or decreasing the formation of inclusion bodies. The addition of sarkosyl may also solubilize many GST-fusion proteins (6).

3.D. Phase 3: Capture of Prey Protein

The protocol provided in Section 4 provides guidelines for performing GST pull-down experiments using MagneGST™ Particles. Depending on the protein pair involved, different degrees of optimization of bait-prey binding conditions may be required. Addition of cofactors, salts or detergents needed for specific protein interactions may be required. The MagneGST™ Binding/Wash Buffer provided is physiologically neutral PBS (see Section 6 for buffer composition) and allows adjustment of binding conditions. In some cases the volume of TnT® reaction and the amount of MagneGST™ Particles used may need optimization. However, be aware that using a large excess of particles may result in non-specific interactions between proteins and the particles.

4. MagneGST™ Pull-Down System Protocol

Materials To Be Supplied By the User
(Solution compositions are provided in Section 6.)

- magnetic separation stand (see Section 2)
- shaker or rotating platform
- radiolabeled methionine (e.g., ^{35}SMet, 10–40µCi per TnT® reaction) if radioactive detection of prey protein is desired or specific antibodies for detection using Western blot analysis
- NANOpure® or double distilled water
- SDS loading buffer
- BSA (Promega Cat.# W3841) or IGEPAL CA-630 (Sigma Cat.# I3021)

The following protocol provides general guidelines for GST pull-down assays using the MagneGST™ Pull-Down System and is based on methods we have developed using a MyoD/GST-Id protein pair. Optimization of protein:protein interaction and washing conditions may be required for each bait-prey pair. The protocol consists of three phases as described in Figure 1. Phases 1 and 2 are performed simultaneously.

4.A. Phase 1: Expression of the Prey Protein in the TnT® T7 Quick Coupled Transcription/Translation Reaction

TnT® T7 Quick Coupled transcription/translation reactions are typically performed in a total volume of 50µl, which generally yields sufficient material for 2 pull-down reactions of 20µl each (GST-fusion protein and GST-only control) and for analysis of transcription/translation efficiency. Because efficiency of transcription/translation and strength of protein:protein interactions may vary for different protein pairs, smaller or larger volumes may be needed. Therefore, the reaction volume may have to be adjusted for your specific protein pair.

Note: In vitro expression of the prey protein can be verified by analyzing 1–5% of the TnT® reaction product on an SDS-PAGE gel, followed by autoradiography or Western blotting with specific antibody.

1. Remove the reagents from storage at –70°C. (Store the RQ1 DNase at –20°C after first use.) Thaw the TnT® T7 Quick Master Mix by hand-warming or on ice. The other components can be thawed at room temperature and stored on ice.
2. Assemble the reaction components as shown in Table 1 using template DNA encoding your prey protein of interest. Incubate the reaction at 30°C for 60–90 minutes. During this incubation period, prepare the MagneGST™ Particles as described in Section 4.B.

   Note: If necessary, the completed TnT® reaction can be stored at 30°C for up to one hour while immobilization of bait proteins onto the MagneGST™ Particles is completed. Storage on ice may result in protein precipitation. However, stability upon storage will vary depending on the particular protein involved. Long-term storage at –70°C is generally preferred over –20°C to help maintain functionality.

Table 1. Example of a TnT® T7 Quick Reaction Using Plasmid DNA.

Components	Reaction Using [^{35}S]Methionine	Reaction Using Unlabeled Methionine
TnT® T7 Quick Master Mix	40µl	40µl
Methionine, 1mM (mix gently prior to use)	—	1µl
[^{35}S]methionine (1,000Ci/mmol at 10mCi/ml)	2µl	—
plasmid DNA template(s) (0.5µg/µl)	2µl	2µl
Nuclease-Free Water to a final volume of	**50µl**	**50µl**

4.B. Phase 2: Immobilization of GST-Fusion Proteins onto MagneGST™ Particles

In Phase 2, MagneGST™ Particles charged with GST-fusion (bait) protein and GST protein (control) are prepared.

We recommend starting with 1ml of bacterial culture containing the GST-fusion protein and 1ml of bacterial culture containing the GST protein as a control. This volume may need to be increased for low-expressing proteins. MagneGST™ Particles alone can also be used as the negative control. We recommend verifying that the GST-fusion protein and the GST protein cultures express comparably by PAGE analysis prior to performing the GST pull-down reactions.

Cell Lysis

1. Harvest cells from 1ml of bacterial culture.

   Note: Freeze-thaw treatment prior to the addition of MagneGST™ Cell Lysis Reagent will increase lysis efficiency for certain strains, such as the BL21 series, and will enhance lysis of cultures grown to a high density. Freezing at −20°C for 15–20 minutes or on dry ice for 5–10 minutes is usually sufficient.

2. Add 200µl of MagneGST™ Cell Lysis Reagent to each fresh or frozen cell pellet. Resuspend the cell pellet at room temperature (20–25°C) by pipetting or gently mixing.

3. Add 2µl of RQ1 RNase-Free DNase.

   Note: Addition of DNase reduces viscosity and can increase the purity of GST-fusion proteins. Up to 5µl of RQ1 RNase-Free DNase can be added to reduce viscosity. The DNase can be omitted, if desired.

4. Incubate the cell suspension at room temperature for 20–30 minutes on a rotating platform or shaker. During this incubation, begin the particle equilibration procedure.

Particle Equilibration

1. Thoroughly resuspend the MagneGST™ Particles by inverting the bottle several times to obtain a uniform suspension.

2. Pipet 20µl of MagneGST™ Particles into a 1.5ml tube.

Do not allow the MagneGST™ Particles to settle for more than a few minutes during capture of the bait protein as this will reduce binding efficiency.

3. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Magnetic capture will typically occur within a few seconds.

4. Carefully remove and discard the supernatant.

5. Remove the tube from the magnetic stand. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles and resuspend by pipetting or inverting.

6. Repeat Steps 3–5 two more times for a total of three washes.

Binding

1. After the final wash, resuspend the particles in 100µl of MagneGST™ Binding/Wash Buffer.

   Note: Addition of up to 1% BSA may reduce nonspecific binding and potential problems with background. IGEPAL CA-630 (NP40 analog) at final concentration 0.5% may have the same effect. The amount of BSA used may need to be optimized for your particular protein.

2. Add 200µl of cell lysate containing the GST-fusion protein or the GST control to the MagneGST™ Particles.

3. Incubate (with constant gentle mixing) for 30 minutes at room temperature on a rotating platform.

Washing

1. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Carefully remove the supernatant and save for gel analysis (optional).

2. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles and gently mix. Incubate at room temperature for 5 minutes while mixing occasionally by tapping or inverting the tube.

3. Place the tube in the magnetic stand and allow MagneGST™ Particles to be captured by the magnet. Carefully remove the supernatant and discard (or save if analysis of wash is desired).

4. Add 250µl of MagneGST™ Binding/Wash Buffer to the particles and mix gently by inverting the tube. (The 5-minute incubation is not required at this wash step.)

5. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Carefully remove the supernatant and discard (or save if analysis of wash is desired).

6. Repeat Steps 4–5 for a total of three washes.

7. After the last wash, resuspend the particles in 20µl of MagneGST™ Binding/Wash Buffer.

   Optional: Remove a 3–5µl aliquot for analysis of the efficiency of immobilization of the GST-fusion protein or GST control onto the particles. To this aliquot, add 20µl 1X SDS loading buffer and elute proteins by boiling for 5 minutes. Analyze by SDS-PAGE.

8. We recommend use of 5µl of the immobilized GST-fusion or GST control for the Phase 3 pull-down assay (Section 4.C). Thus, 20µl of particles will provide sufficient material for more than one pull-down assay. However, in some cases more than 5µl may be required for one pull-down reaction.

4.C. Phase 3: Capture, Wash and Analysis of Prey Protein Capture

1. To each 5µl aliquot of particles carrying GST-fusion protein (or GST control) add 20µl of the TnT® T7 Quick coupled transcription/translation reaction from Phase 1.
2. Add 155µl MagneGST™ Binding/Wash Buffer and 20µl 10% BSA (or 175µl MagneGST™ Binding/Wash Buffer if BSA is omitted) to a final volume of 200µl for each pull-down reaction. (Addition of 1% BSA may reduce non-specific binding and potential problems with background. IGEPAL CA-630 [NP40 analog] at a final concentration of 0.5% may have the same effect. The amount of BSA or IGEPAL used may require optimization for your particular protein:protein interaction.)

   Note: MagneGST™ Binding/Wash Buffer is a neutral PBS buffer, allowing the user to optimize buffer conditions for each specific protein:protein interaction. Some protein interactions will require the presence of various cofactors, salts and detergents.
3. Incubate for 1 hour (with gentle mixing) at room temperature on a rotating platform.

   Note: Briefly vortexing at the end of this incubation period may help remove non-specific adherent proteins and reduce background. A different incubation temperature and time may be required for your specific protein: protein interaction.
4. Place the tube in a magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Carefully remove the supernatant and save for analysis (optional).

Washing

The stability of different protein:protein interactions is protein pair-specific and depends on the K_d of the interaction. Optimization of washing conditions may be required for less stable interactions. For example, the number of washes and the volume of each wash may need to be changed.

1. Add 400µl of MagneGST™ Binding/Wash Buffer, and mix gently by inverting the tube.
2. Incubate at room temperature for 5 minutes while mixing occasionally by tapping or inverting the tube.
3. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Remove the supernatant and save for analysis (it is especially important to keep this fraction during initial optimization).
4. Add 400µl of MagneGST™ Binding/Wash Buffer and mix gently by inverting the tube. (The 5-minute incubation is not required at this wash step.)
5. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Carefully remove the supernatant and save for analysis (optional).
6. Repeat Steps 4 and 5 three more times for a total of five washes.

Elution

1. Add 20µl of 1X SDS loading buffer.
2. Incubate for 5 minutes at room temperature with mixing.
3. Place the tube in the magnetic stand and allow the MagneGST™ Particles to be captured by the magnet. Remove the eluate for analysis.

Analysis

Prepare samples for SDS-PAGE analysis. For radioactively labeled prey proteins, we recommend loading 1–2 % of each sample volume. For ease of handling, first dilute the eluted sample 1:10 in SDS loading buffer (e.g., 2µl eluted sample + 18µl 1X SDS loading buffer). Boil the diluted sample for 5 minutes then load 2–4µl onto an SDS-PAGE gel. For Western blot analysis, we suggest loading 25–50% of undiluted eluted sample (after boiling) onto a SDS-PAGE gel. Stronger signals in the experimental samples compared to the GST control lanes indicate that the prey is specifically pulled down by the bait protein.

5. 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**

Phase 1

Symptoms	Causes and Comments
Low or no translation efficiency	Ethanol or salt may inhibit translation. Remove residual ethanol or salt from template DNA.
	Incubation at 37°C decreases protein synthesis. Incubate TnT® reaction at 30°C.
Unexpected bands present in the translation reaction products	Denature sample at a lower temperature (e.g., 60–80°C) for 10–15 minutes before loading on gel.
	Add protease inhibitors to prevent proteolysis of translation product.

5. Troubleshooting (continued)

Phase 2

Symptoms	Causes and Comments
Inefficient immobilization of GST fusion protein onto MagneGST™ Particles	Expressed protein unstable. Add protease inhibitors during cell lysis step.
	Change induction conditions.
	Binding of GST-fusion proteins is pH sensitive; pH above 8.0 may interfere with efficient binding.
	Increase the volume of the MagneGST™ Binding/Wash Buffer during immobilization to optimize binding conditions.
	Lowering the temperature (4°C) may increase binding efficiency.
	Addition of DTT to a final concentration of 5mM prior to cell lysis has been shown to increase binding of some GST-fusion proteins (6).
	Limit the number or duration of washes during immobilization.

Phase 3

Symptoms	Causes and Comments
Interacting protein not detected	Weak bait-prey interaction. Addition of cofactors may be required to optimize and stabilize the bait-prey interaction.
	Reduce stringency of washing.
	Low expression of prey protein in TnT® reaction. Add more of the TnT® reaction product to the pull-down experiment.
High background or many contaminating bands (proteins detected in GST control)	Nonspecific interactions. Increase stringency of washing. Increase number and volume of washes. Include salt or detergent in the MagneGST™ Binding/Wash Buffer.
	Add BSA (up to 1% final concentration) to the MagneGST™ Particles during GST-fusion protein immobilization and prey protein capture. Addition of IGEPAL-CA630 (NP 40 analog) at 0.5% final concentration may also help reduce background.
	Too many MagneGST™ Particles in pull-down reaction. Reduce the number of particles used in the pull-down reaction.

6. Composition of Buffers and Solutions

1X SDS gel-loading buffer

50mM	Tris-HCl (pH 6.8)
2%	SDS
0.1%	bromophenol blue
10%	glycerol
10mM	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.

MagneGST™ Binding/Wash Buffer (pH 7.2)

4.2mM	Na_2HPO_4
2mM	KH_2PO_4
140mM	NaCl
10mM	KCl

7. References

1. Kaelin, W.G. *et al.* (1991) Identification of cellular proteins that can interact specifically with the T/E1A-binding region of the retinoblastoma gene product. *Cell* **64**, 521–32.
2. Golemis, E. (2002). *A Molecular Cloning Manual: Protein-Protein Interactions*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
4. Hurst, R. *et al.* (1996) The TnT® T7 Quick Coupled Transcription/Translation System. *Promega Notes* **58**, 8–11.
5. Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual, 3rd edition*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
6. Frangioni, J.V. and Neel, B.G. (1993) Solubilization and purification of enzymatically active glutathione S-transferase (pGEX) fusion proteins. *Anal. Biochem.* **210**, 179–87.

8. Related Products

Product	Size	Cat.#
MagneGST™ Protein Purification System	40 reactions	V8600
	200 reactions	V8603
Anti-Mouse IgG (H+L), AP Conjugate	100µl	S3721
Anti-Rabbit IgG (Fc), AP Conjugate	100µl	S3731
Anti-Human IgG (H+L), AP Conjugate	100µl	S3821
Anti-Rat IgG (H+L), AP Conjugate	100µl	S3831
Donkey Anti-Goat IgG, AP	60µl	V1151
Blot-Qualified BSA	10g	W3841
Tween® 20	2.5ml	W3831
Western Blue® Stabilized Substrate for Alkaline Phosphatase	100ml	S3841

9. Summary of Changes

The following changes were made to the 5/17 revision of this document:

1. Removed expired patent statements.

[a]Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

[b]This product is licensed for use under U.S. Pat. No. 6,174,704.

[c]Patent Pending.