Compatibility of Single Step (KRX) Competent Cells with the MagneGST™ Pull-Down System

ABSTRACT This study demonstrates the use of Single Step (KRX) Competent Cells for studying protein:protein interactions using the MagneGST™ Pull-Down System. GST-fusion bait protein expressed in KRX was captured on MagneGST™ glutathione-linked magnetic particles and incubated with prey protein expressed in vitro in the TNT® T7 Quick Coupled Transcription/Translation System. Captured prey was detected using HaloTag® Interchangeable Labeling Technology.

Trista Schagat, Ph.D., Promega Corporation

Single Step (KRX) Competent Cells are designed for efficient transformation and tightly controlled protein expression, making them ideal for protein:protein interaction studies.

INTRODUCTION

The MagneGST™ Pull-Down System (Cat.# V8870) is designed for detection and screening of protein:protein interactions (1). Bait protein (protein of interest fused to glutathione-S-transferase [GST]) is expressed in *E. coli*, then immobilized from lysates on glutathione-linked magnetic particles. Prey protein is synthesized in vitro using the TNT® T7 Quick Coupled Transcription/Translation System (Cat.# L1170). Prey is captured directly from the TNT® reaction on the magnetic particles carrying the bait protein (Figure 1). Pull-down reactions are processed rapidly using a magnetic stand.

Single Step (KRX) Competent Cells[a] (Cat.# L3001) are ideal for rapid generation of clones and protein expression. They are designed for efficient transformation, blue/white selection, and tightly controlled recombinant protein expression via a T7 promoter (2). This makes KRX cells ideal for protein:protein interaction studies, which often involve generating numerous constructs for expression of truncated or mutated proteins.

As we have previously described, a significant amount of protein can be expressed in KRX cells using a short 4-hour induction protocol (3). Here we use this short induction protocol to express bait protein for analysis in the MagneGST™ Pull-Down System. For rapid, non-radioactive detection of the prey we used the HaloTag® Interchangeable Labeling Technology.

MAGNEGST™ PULL-DOWN METHOD WITH SINGLE STEP (KRX) COMPETENT CELLS

pFN2A (GST) Flexi® Vector containing the protein of interest coding region or no insert (pFN2K (GST) Flexi® Vector with barnase gene removed) was transformed into Single Step (KRX) Competent Cells and induced following the standard protocol in the *Single Step (KRX) Competent Cells Technical Bulletin* #TB352. KRX cultures were collected after a 4-hour induction and processed to purify the bait protein following the protocol described in the *MagneGST™ Pull-Down System Technical Manual* #TM249.

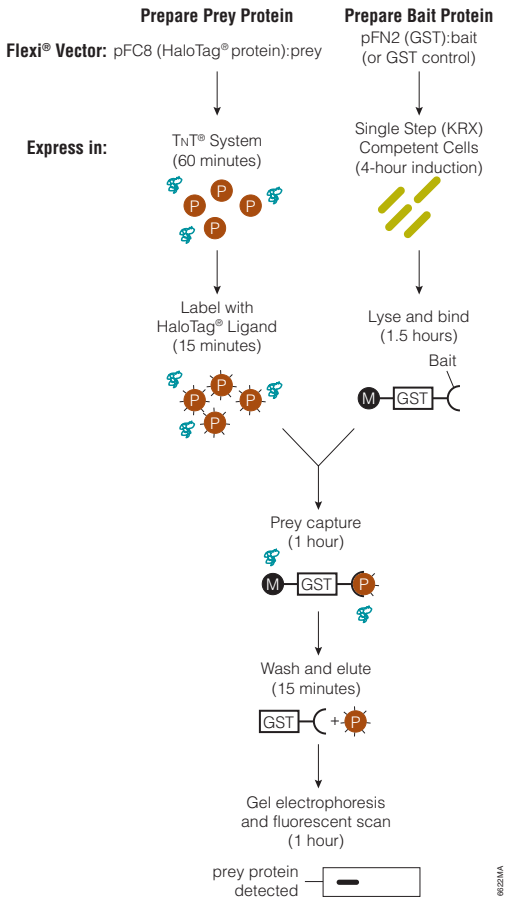

Figure 1. Experimental overview. Bait protein (GST fused to the protein of interest) was expressed in Single Step (KRX) Competent Cells for analysis of protein:protein interactions in the MagneGST™ Pull-Down System. HaloTag® Interchangeable Labeling Technology was used for rapid non-radioactive detection of captured prey. P = Prey Protein, M = MagneGST™ Particles.

Prey protein was expressed in vitro as a HaloTag® fusion protein using the T7 promoter in the pFC8A

(HaloTag®) CMV Flexi® Vector. After expression, the HaloTag® fusion proteins were fluorescently labeled with the HaloTag® TMR Ligand (Cat.# G8251). Pull-down reactions were performed as described in the *MagneGST™ Pull-Down System Technical Manual* #TM249.

DETECTING PROTEIN:PROTEIN INTERACTIONS

FK506 binding protein 12 (FKBP) and Frb (the FKBP-rapamycin binding domain of FKBP-rapamycin-associated protein) form a ternary complex in the presence of the immunosuppressant compound, rapamycin (4). Figure 2 shows the rapamycin-dependent interaction of FKBP with Frb. The GST-FKBP fusion and GST alone expressed well in KRX cells within the 4-hour induction period and yielded sufficient protein for loading on the MagneGST™ magnetic particles and analysis in the pull-down reactions. No detectable Frb was captured by the GST-only particles or the GST-FKBP-loaded particles in the absence of rapamycin. Fusing the HaloTag® protein with Frb did not interfere with the Frb-FKBP-rapamycin interaction and allowed simple, rapid fluorescent analysis.

SUMMARY

This study demonstrates the compatibility of the Single Step (KRX) Competent Cells with the MagneGST™ Pull-Down System. The rapamycin-dependent FKBP:Frb protein interaction was successfully detected using the HaloTag® Interchangeable Labeling Technology for non-radioactive detection. The Single Step (KRX) Competent Cells are designed for efficient transformation and tightly controlled protein expression, making them a convenient, expeditious choice for protein:protein interaction studies.

ACKNOWLEDGMENTS

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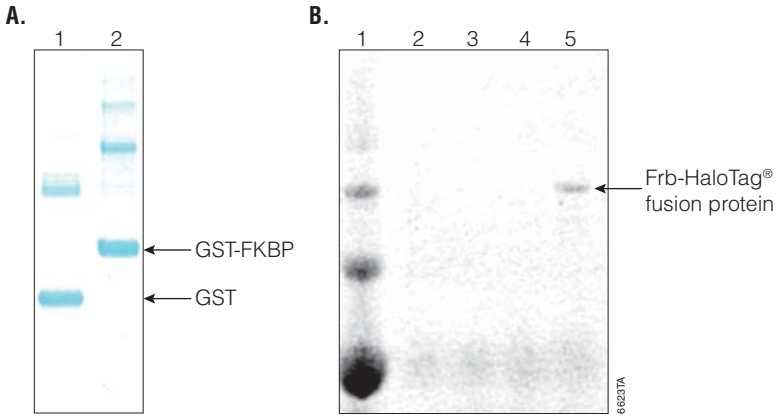

Figure 2. Non-radioactive detection of FKBP:Frb:rapamycin interaction using KRX cells with the MagneGST™ Pull-Down System. Pull-down experiments were performed as described in Technical Manual #TM249 and included 1% BSA. Samples were run on 4–12% Novex NuPAGE® Bis-Tris gels. **Panel A.** Five microliters of particles charged with GST only (lane 1) or GST-FKBP (lane 2) from a 4-hour induction in KRX cells and visualized by SimplyBlue™ Safestain (Invitrogen). **Panel B.** Frb was expressed as a fusion with HaloTag® protein and labeled after translation with the HaloTag® TMR Ligand (1µM final concentration, incubated for 15 minutes at room temperature). Results were detected using a Hitachi FMBIO® II instrument at an excitation wavelength of 505nm. Lane 1, 2% of the TNT® reaction; lane 2, 50% of the GST-only pull-down reaction; lane 3, 50% of the GST-only pull-down reaction in the presence of 2µM rapamycin; lane 4, 50% of the GST-FKBP control reaction (pull-down in the absence of rapamycin); lane 5, 50% of the GST-FKBP pull-down reaction in the presence of 2µM rapamycin.

PROTOCOLS

- *Single Step (KRX) Competent Cells Technical Bulletin* #TB352, Promega Corporation
  www.promega.com/tbs/tb352/tb352.html
- *MagneGST™ Pull-Down System Technical Manual* #TM249, Promega Corporation
  www.promega.com/tbs/tm249/tm249.html

ORDERING INFORMATION

Product	Size	Cat.#
Single Step (KRX) Competent Cells	5 × 200µl	L3001
	20 × 50µl	L3002

[a] Usage restrictions apply to Bacterial Strains JM109(DE3), BL21(DE3)pLysS and KRX and to any derivatives thereof. Please read the statement on page 26 describing these restrictions before purchasing any of these products.