INSTRUCTIONS

Pierce® Glutathione Magnetic Beads

88821 88822

2076.1

Number	Description
88821	**Pierce Glutathione Magnetic Beads**, 4 ml, supplied as a 25% slurry in 20% ethanol
88822	**Pierce Glutathione Magnetic Beads**, 20 ml, supplied as a 25% slurry in 20% ethanol

Storage: Upon receipt store at 4°C. Products are shipped with an ice pack.

Introduction

The Thermo Scientific Pierce Glutathione Magnetic Beads provide a fast and convenient method for manual or automated purification of glutathione-S-transferase- (GST) fusion proteins from a bacteria, yeast or mammalian crude cell lysate. These beads are simply added to a lysate containing the GST-fusion protein. After incubation, non-bound proteins are removed by washing, and the GST-fusion proteins are eluted using reduced glutathione. A magnetic stand is used for manual processing. For automated processing, the Thermo Scientific KingFisher® Flex or KingFisher 96 Instrument is used. These instruments are especially useful for large-scale screening of multiple samples.

Table 1. Characteristics of Thermo Scientific Pierce Glutathione Magnetic Beads.

Composition:	iron oxide particles with reduced glutathione (GSH) covalently attached to the surface and encapsulated by crosslinked agarose
Magnetization:	Superparamagnetic (no magnetic memory)
Mean Diameter:	1-10 µm
Concentration:	25% slurry (based on volume) in 20% ethanol
Binding Capacity:	5-10 mg of GST fusion protein per 1 ml of settled beads (i.e., 4 ml of 25% slurry)

Important Product Information

- Do not freeze, dry or centrifuge the Pierce Magnetic Beads, which causes bead aggregation and loss of binding activity.
- Cell lysates can be prepared from a variety of methods, including Thermo Scientific Cell Lysis Reagents (e.g., B-PER® Bacterial Protein Extraction Reagent, Product No. 78243, 78248; Y-PER® PLUS Dialyzable Yeast Protein Extraction Reagent, Product No. 78999; M-PER® Mammalian Protein Extraction Reagent, Product No. 78501), sonication and French press.
- For best results, perform a small-scale test extraction to estimate the expression level and determine the solubility of the GST-fusion protein. After lysis, centrifuge and analyze supernatant by SDS-PAGE. Only soluble protein extracts that are clarified and have no protein particulates are successfully processed with the Pierce Glutathione Magnetic Beads.
- Inclusion bodies of GST-fusion proteins that have been solubilized in urea or guanidine must be dialyzed to remove the denaturant(s) and refold the protein before affinity purification with Pierce Glutathione Magnetic Beads. (GST must be functional to bind glutathione.)
- To minimize protein degradation, include protease inhibitors (e.g., Thermo Scientific Halt Protease Inhibitor Single-Use Cocktail EDTA-free, Product No. 78425) in cell lysate preparations.
- Boiling the beads in SDS-PAGE sample buffer is acceptable for single-use applications. Boiling will cause bead aggregation and loss of binding activity.

Procedure for Manual GST-Fusion Protein Purification from Bacteria

A. Additional Materials Required

- 1.5 ml microcentrifuge tubes
- Bacterial cell lysate
- Binding/Wash Buffer: 125 mM Tris, 150 mM NaCl, pH 8.0
- Elution Buffer: Immediately before use prepare 50 mM reduced glutathione (Product No. 78259) in Binding/Wash Buffer
- Magnetic stand (e.g., Thermo Scientific MagnaBind Magnet for 6 × 1.5 ml Microcentrifuge Tubes, Product No. 21359)

B. Pre-wash the Pierce Glutathione Magnetic Beads

Note: To ensure bead homogeneity, mix the vial thoroughly before use by repeated inversion, gentle vortexing or rotating platform.

1. Place 100 µl of Pierce Glutathione Magnetic Beads into a 1.5 ml microcentrifuge tube.
2. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
3. Add 300 µl of Binding/Wash Buffer to the tube. Invert the tube several times or vortex gently to mix. Collect the beads with a magnetic stand, then remove and discard the supernatant. Repeat this step twice.

   Note: Do not allow the beads to dry. If necessary, store the beads in Binding/Wash buffer before proceeding with purification protocol.

C. GST-Fusion Protein Purification from a Bacterial Cell Lysate

Note: The procedure is for 50 µl of cell lysate prepared from 1 ml of bacterial culture. Adjust the buffer and bead quantities as needed for the specific lysate.

1. Dilute 50 µl of bacterial cell lysate with 250 µl of Binding/Wash Buffer.
2. Add diluted lysate to the tube containing pre-washed magnetic beads from above and gently invert or vortex to mix.
3. Incubate the samples at room temperature with mixing (e.g., on a rocking platform) for 1 hour. Alternatively, incubate at 4˚C.
4. Collect the beads with a magnetic stand and remove the supernatant. If desired, save the supernatant for analysis.
5. Add 300 µl of Binding/Wash Buffer to the tube, mix well, collect the beads with a magnetic stand and discard the supernatant. Repeat this wash twice.
6. Add 200 µl of Elution Buffer to the tube, mix well and incubate 5 minutes at room temperature with mixing.

   Note: If desired, smaller elution volumes can be used.

   Note: If the elution is not complete, perform an additional 5 minute elution. Combine the eluates from multiple elutions.

Procedure for Automated GST-Fusion Protein Purification from Bacteria

A. Additional Materials Required

- KingFisher Flex with 96 deep well head (Product No. 5400630) or KingFisher 96 (Product No. 5400500)
- Microtiter Deep Well 96 Plate, V-bottom, polypropylene (100-1,000 µl; Product No. 95040450)
- KingFisher Flex 96 tip comb for Deep Well magnets (Product No. 97002534)
- Bacterial Cell Lysate
- Binding/Wash Buffer: 125 mM Tris, 150 mM NaCl, pH 8.0
- Elution Buffer: Immediately before use prepare 50 mM reduced glutathione (Product No. 78259) in Binding/Wash Buffer

B. Preparation of the KingFisher Instrument and Plate Set-up

Note: The following protocols are designed for general use with the KingFisher Flex or KingFisher 96. The protocol can be modified according to customer needs using the BindIt™ Software provided with the instrument.

1. Download the "GST-Fusion Protein Purification" protocol from the web site (*www.thermo.com/kingfisher*) into the BindIt Software on an external computer.
2. Transfer the protocol to the KingFisher Flex or KingFisher 96 from an external computer. See the BindIt Software User Manual for detailed instructions on importing protocols.
3. Set up the plates according to Table 2.

Table 2. Pipetting instructions for the automated purification protocol.

Plate #	Plate Name	Plate Type	Content	Volume
1	Beads	Microtiter Deep Well 96 Plate	Glutathione beads Binding/Wash Buffer	100 µl 100 µl
2	Bead Prewash	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	1,000 µl
3	Protein Sample	Microtiter Deep Well 96 Plate	Bacterial Cell Lysate Binding/Wash Buffer	50 µl 250 µl
4	Wash 1	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
5	Wash 2	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
6	Wash 3	Microtiter Deep Well 96 Plate	Binding/Wash Buffer	300 µl
7	Elution	Microtiter Deep Well 96 Plate	Elution Buffer	200 µl
8	Tip Plate	Microtiter Deep Well 96 Plate	KingFisher Flex 96 tip comb for Deep Well magnets	-

Notes:

- If using less than 96 wells, fill the same wells in each plate. For example, if using wells A1 through A12, use these same wells in all plates.
- To ensure bead homogeneity, mix the vial thoroughly by repeated inversion, gentle vortexing or rotating platform before adding the beads to Plate 1.
- Combine the Tip Comb with a Deep Well 96 plate. See the instrument user manual for detailed instructions.

C. Executing the GST-Fusion Protein Purification Protocol on the KingFisher Flex or KingFisher 96

1. Select the protocol using the arrow keys in the instrument keypad and press *Start*. See the instrument user manual for detailed information.
2. Slide open the door of the instrument's protective cover. Load the plates into the instrument according to the protocol request, placing each plate in the same orientation. Confirm each action by pressing *Start*.
3. After the samples are processed, remove the plates as instructed by the instrument's display. Press *Start* after removing each plate. Press *Stop* after all plates are removed.

General Troubleshooting

Problem	Possible Cause	Solution
Low protein recovery	Proteolysis of sample	Add protease inhibitors
	Not enough magnetic beads used	Increase the amount of magnetic beads
	Insufficient target protein present in sample	Increase sample size
GST-fusion protein does not elute	Large proteins elute less efficiently and/or elution conditions are too mild	Increase incubation time with Elution Buffer or increase ionic strength of Elution Buffer by adding NaCl to a final concentration of 100-500 mM
		Make sure the pH of the Elution Buffer is ≥ 8.0
		Include 0.1%-1% Triton® X-100 or Tween®-20 Detergent in the Elution Buffer
		Increase the volume of the Elution Buffer or perform two elution steps
Multiple, nonspecific bands appear in eluted sample	Nonspecific protein binding to the magnetic beads	Add NaCl to the Binding/Wash Buffer up to a final concentration of 0.5 M to increase stringency
Magnetic beads aggregate	Magnetic beads were frozen	Handle the beads as directed in the instructions
	Buffer is incompatible with magnetic beads	

Frequently Asked Questions for the KingFisher Instruments

Question	Answer
Which plates are compatible with KingFisher Flex and KingFisher 96?	The KingFisher Flex is compatible with the KingFisher 24 Deep Well plates, Microtiter 96 Deep Well Plates, KingFisher 96 plates and 96 PCR plates. The KingFisher 96 is compatible with the Microtiter 96 Deep Well Plates, KingFisher 96 Plates and 96 PCR plates.
Is it possible to concentrate samples during the run?	Both deep well plates and KingFisher 96 plates can be used during the same run. Therefore, it is possible to start the processing by using larger volumes (in a deep well plate) and elute the purified sample to a smaller volume (in a KingFisher 96 plate).
Is it possible to heat the samples during the run?	The heating block is located inside the instrument and can be used automatically during the sample process. All plates compatible with the KingFisher Flex can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells or the eluted proteins sticks to the wells?	Proteins conjugated to beads and eluted proteins can nonspecifically bind to plastics. Adding detergent in the Binding/Wash Buffer prevents the protein conjugated to the beads from sticking (0.05%-0.1% Tween-20 Detergent). Also include a small amount of detergent in the Elution Buffer (e.g., 0.05% Tween-20 Detergent) or silanize the elution plate.
Are the reagent volumes in each well critical?	For best results, keep the specified volumes within defined limits to avoid spillover.

Additional Information

- **Visit *www.thermo.com/pierce* for additional information including the following:**
  - Tech Tip protocol: Protein Stability and Storage

- **Visit *www.thermo.com/kingfisher* for information on KingFisher Products.**

- **In the U.S.A., purchase KingFisher Supplies from VWR. Contact your local Thermo Fisher Scientific office to purchase KingFisher Supplies outside the U.S.A.**

Related Products

24615	**Imperial™ Protein Stain**, 1 L, sufficient for up to 50 mini gels
25200-25244	**Precise™ Protein Gels**, see catalog or web site for a complete listing
88800, 88801	**Pierce Protein A Magnetic Beads,** 1 ml, 5 ml
88806, 88807	**Pierce Protein G Magnetic Beads,** 1 ml, 5 ml
88811, 88812	**Pierce Magnetic TiO_2 Phosphopeptide Enrichment Kit,** 96 samples, 24 samples

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Current versions of product instructions are available at *www.thermo.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | www.thermo.com/pierce