Pierce™ Glutathione Magnetic Agarose Beads

Catalog Numbers 78601 and 78602

Doc. Part No. 2162619 **Pub. No.** MAN0015958 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Thermo Scientific™ Pierce™ Glutathione Magnetic Agarose Beads (Table 1) provide a fast, convenient method for purification of glutathione-S-transferase (GST) from a bacterial, yeast, or mammalian crude cell lysate. Pierce™ Glutathione Magnetic Agarose Beads are well suited for purifying GST fusion proteins from a soluble protein extract and are ideal for the purification of proteins expressed at low levels from diluted supernatants. The beads can be used in manual applications with a magnetic stand (e.g., Thermo Scientific™ DynaMag™-2 Magnet; Product No. 12321D) or automated applications with an instrument such as the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor. Automated instruments are especially useful for higher throughput purification and screening of purification conditions.

Table 1 Characteristics of the Thermo Scientific™ Pierce™ Glutathione Magnetic Agarose Beads.

Composition	Glutathione covalently attached to magnetite-embedded 6% agarose beads
Magnetization	Ferrimagnetic with low remanence
Mean diameter	10-40 µm
Bead concentration	25% slurry in 20% ethanol
Binding capacity	≥10 mg GST/mL settled beads

Contents

Contents	Cat. No. 78601	Cat. No. 78602	Storage
Pierce™ Glutathione Magnetic Agarose Beads	4 mL, supplied at 25% v/v suspension in 20% ethanol	20 mL, supplied at 25% v/v suspension in 20% ethanol	Store at 4°C.

Additional information

- Do not centrifuge, dry or freeze the beads. Handling the beads in this way will cause the beads to aggregate and lose binding capacity.
- Cell lysates can be prepared from a variety of methods, including Thermo Scientific™ Cell Lysis Reagents (e.g., B-PER™ Complete Bacterial Protein Extraction Reagent , Product No. 89821, 89822; Y-PER™ PLUS Dialyzable Yeast Protein Extraction Reagent, Product No. 78999; M-PER™ Mammalian Protein Extraction Reagent, Product No. 78501), sonication and French press.
- To minimize protein degradation, include protease inhibitors (e.g., Halt™ Protease Inhibitor Cocktail, EDTA-free (100X), Product No. 78425) in cell lysate preparations.
- Protein yield and purity are dependent upon the expression level, conformation and solubility characteristics of the recombinant fusion protein; therefore, it is important to optimize these parameters. For best results, perform a small-scale test to estimate the expression level and determine the solubility of each GST-tagged protein.
- Concentration of proteins in the eluted fractions can be determined by using the Thermo Scientific™ Pierce™ 660nm Protein Assay Kit (Product No. 22662) or Detergent Compatible Bradford (Product No. 23246).

Manual purification of GST-fusion proteins

Materials required but not supplied for manual purification

- 1.5 mL low protein binding microcentrifuge tubes (Product No. 90410)
- Cell lysate/sample containing GST fusion protein

  Note: Samples can be concentrated using Thermo Scientific™ Pierce™ Protein Concentrators (thermofisher.com/concentrators).
- Magnetic stand (e.g., Thermo Scientific™ DynaMag™–2 magnet; Product No. 12321D)
- Nutating mixer (e.g., Thermo Scientific™ HulaMixer™ Sample Mixer; Product No. 15920D)
- **Equilibration/Wash Buffer: 125 mM Tris-HCl, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, pH7.4**

  Note: Add DTT and EDTA immediately before use.
- **Elution Buffer: 50 mM glutathione, reduced (Product No. 78259) in Equilibration/Wash Buffer, pH 7.4**

  Note: Make new Elution Buffer before use and readjust pH after adding glutathione.

Perform manual purification of GST-fusion proteins

Required bead volume can vary with the amount of protein in a sample and the magnetic separator used. The protocols listed below are designed for the purification of ~0.1-0.25 mg of recombinant GST-tagged protein. Adjust volumes accordingly for smaller or larger scale purifications.

Binding capacity	Settled bed volume
0.3 mg	25 µL
1 mg	100 µL
2.5 mg	250 µL
5 mg	500 µL
10 mg	1000 µL

1. Place 100 µL bead slurry (25µL settled beads) into a 1.5 mL microcentrifuge tube.
2. Add 400 µL of Equilibration/Wash Buffer to the beads and vortex for 10 seconds to mix.
3. Place the tube into a magnetic stand to collect the beads against the side of the tube. Remove and discard the supernatant.
4. Add 500 µL of Equilibration/Wash Buffer to the tube. Vortex the beads for 10 seconds and collect the beads with a magnetic stand. Remove and discard the supernatant.

   Note: Do not allow the beads to dry. If necessary, store the beads in Equilibration/Wash Buffer before proceeding with the purification protocol.
5. Add DTT and EDTA (1 mM final concentration each) to the GST fusion protein lysate before diluting with Equilibration/Wash Buffer. Prepare 500 µL final volume of sample by diluting 250 µL protein extract with an equal volume of Equilibration/Wash Buffer.

   Note: Reserve a small volume of load for downstream analysis by SDS-PAGE.
6. Add 500 µL of prepared protein extract to the washed beads, vortex for 10 seconds and then mix on an end-over-end rotator for 1-2 hours.
7. Collect the beads by placing the tube on a magnetic stand. If desired, save the supernatant (flow-through) for downstream analysis.
8. Add 500 µL of Wash Buffer to the tube and mix well. Collect the beads with a magnetic stand, then remove and discard the supernatant or reserve in separate tube for downstream analysis if necessary.
9. Repeat wash step.
10. Add 250 µL of Elution Buffer to the tube and then mix on an end-over-end rotator for 10 minutes.
11. Collect beads on a magnetic stand. Carefully remove and save the supernatant containing the GST fusion protein.

    Note: Smaller elution volumes can be used, but elution efficiency will be lowered.
12. Repeat the elution steps (steps 10-11) using 250 µL of Elution Buffer. Incubate the beads for 10 minutes.
13. Monitor the elution for protein content using the Pierce™ Detergent Compatible Bradford Assay (Product No. 23246) or Pierce™ 660nm Protein Assay Kit (Product No. 22662). Eluted protein can also be directly analyzed by SDS-PAGE.

Automated purification of GST-fusion proteins

Materials required but not supplied for automated purification

- KingFisher™ Flex Magnetic Particle Processor with 96 Deep-Well Head (Product No. 5400630)
- KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene (100-1000 µL; Product No. 95040450)
- 96 Deep-Well Tip Combs for KingFisher™ Flex Magnetic Particle Processor (Product No. 97002534)
- **Equilibration/Wash Buffer: 125 mM Tris-HCl; 150 mM NaCl; 1mM DTT; 1 mM EDTA, pH 7.4**

  Note: Add DTT and EDTA immediately before use.
- **Elution Buffer: 50 mM glutathione, reduced (Product No. 78259) in Equilibration/Wash Buffer, pH 7.4**

  Note: Make new buffer before use and readjust pH after adding glutathione.

Prepare instrument and set up plates

Note: The following protocol is designed for use with the KingFisher™ Flex Magnetic Particle Processor. The protocol can be modified according to customer needs using the Thermo Scientific™ BindIt™ Software provided with the instrument.

1. Download the appropriate BindIt™ Software protocol from the product page (Product No. 78601, 78602) on the Thermo Fisher Scientific website into the BindIt™ Software on an external computer.
2. Transfer the protocol to the KingFisher™ Flex instrument from an external computer. See the BindIt™ Software user manual for detailed instructions on importing protocols.
3. Set up plates according to Table 2.

Table 2 Plate set-up.

Plate #	Plate name	Content	Volume
1	Beads	Beads	100 µL
		Equilibration Buffer	400 µL
2	Bead equilibration	Equilibration Buffer	500 µL
3	Bind	Protein in Equilibration Buffer	500 µL
4	Wash 1	Wash Buffer	500 µL
5	Wash 2	Wash Buffer	500 µL
6	Elution 1	Elution Buffer	250 µL
7	Elution 2	Elution Buffer	250 µL
8	Tip plate	KingFisher™ 96 tip comb for DW magnets	—

Note:

- If fewer than 96 wells are used, 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.
- A minimum volume of 100 µL is required for efficient elution of bound protein.

Perform GST-fusion purification protocol on the Kingfisher Flex instrument

1. Select the protocol using the arrow keys on the instrument keypad and press **Start**. See the KingFisher™ Flex Magnetic Particle Processor Instrument user manual for detailed information.
2. Slide open the door of the instrument's protective cover.
3. Load plates into the instrument according to the protocol requests, placing each plate in the same orientation. Confirm each action by pressing **Start**.
4. After sample processing, remove the plates as instructed by the instrument's display. Press **Start** after each plate. Stop after removing all of the plates.

Frequently asked questions for the Kingfisher instruments

Question	Answer
Which plates are compatible with the KingFisher™ Flex Magnetic Particle Processor?	The KingFisher™ Flex Magnetic Particle Processor is compatible with the KingFisher™ Flex 24 Deep-Well Plates, KingFisher™ Deepwell 96 Plate, V-bottom, polypropylene, KingFisher™ 96 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 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 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 Magnetic Particle Processor can be heated using specially designed, interchangeable heating blocks.
Why do the beads stick to the plastic tips and wells? Why does the eluted protein stick to the wells?	Eluted proteins and proteins conjugated to beads can nonspecifically bind to plastics. Adding detergent to Binding/Wash Buffer prevents the protein conjugated to the bead from sticking (0.05%-0.1% Tween™-20 Detergent). Also include a small amount of detergent in the elution buffer 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.

Related products

Product	Cat. no.
Pierce™Glutathione Superflow Agarose	25236
Pierce™ Glutathione Chromatography Cartridges	16109
B-PER™ Bacterial Protein Extraction Reagent	78248
Halt™ Protease Inhibitor Cocktail, EDTA-free (100X)	78425
Zeba™ Spin Desalting Columns, 7K MWCO	89892
Pierce™ Detergent Compatible Bradford Assay	23246
Pierce™ 660nm Protein Assay Kit	22662
B-PER™ Complete Bacterial Protein Extraction Reagent	89821
B-PER™ with Enzymes Bacterial Protein Extraction Kit	90078
Slide-A-Lyzer™ Dialysis Cassettes	See thermofisher.com for product line.
Pierce™ Protease and Phosphatase Inhibitor Tablets	
Novex™ WedgeWell™ Tris-Glycine Mini Gels	

Troubleshooting

Observation	Possible cause	Recommended action
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.
		Ensure the Elution Buffer is new and has a pH ≥8.0.
		Include 0.1-1% Triton™ X-100 Detergent or Tween™-20 Detergent in the Elution Buffer.
		Increase the Elution Buffer volume or perform additional elution steps.
Low protein yield.	Proteolysis of sample.	Add protease inhibitors.
	Insufficient amount of magnetic beads.	Increase amount of magnetic beads.
	Insufficient target protein present in sample.	Increase sample size.
Multiple nonspecific bands appear in the eluted sample.	Nonspecific protein binding to the magnetic beads.	Add NaCl to the Equilibration/Wash Buffer to a final concentration of 0.5 M to increase stringency.
Magnetic beads aggregate.	Magnetic beads were frozen.	Handle the beads as directed in instructions.
	Buffer was incompatible with magnetic beads.	Handle the beads as directed in instructions.

Limited product warranty

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Revision history: Pub. No. MAN0015958

Revision	Date	Description
A.0	15 August 2016	New manual

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