

Product Name	Cat. No.	Pack Size
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<b>Glutathione Agarose Beads</b>	# BB-GA004A	1 ml Packed Bead Volume
	# BB-GA004B	5 ml Packed Bead Volume
	# BB-GA004C	10 ml Packed Bead Volume
	# BB-GA004D	25 ml Packed Bead Volume
	# BB-GA004E	50 ml Packed Bead Volume

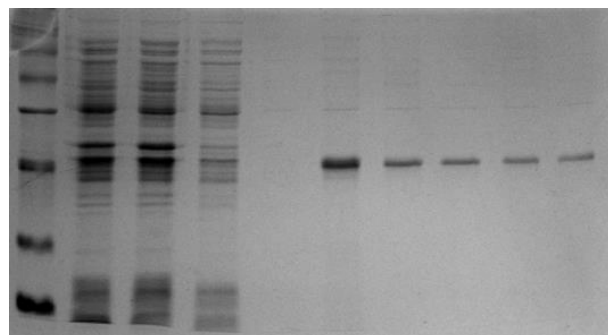
Glutathione Agarose bead is designed for high capacity single-step affinity purification of Glutathione S-Transferase (GST) tagged fusion proteins. This product consists of reduced glutathione attached to epoxy activated 4% cross-linked beaded agarose through the sulfur atom with a 12 atom (10 carbon) spacer arm. This 12 atom spacer arm between bead and the ligand allows strong affinity interaction between glutathione & GST part of fusion protein and minimizes steric inhibition.

Affinity chromatography using glutathione-agarose permits rapid, mild, non-denaturing, and highly selective purification of glutathione binding enzymes such as GST, glutathione peroxidase, and glyoxalase I. GST-tagged fusion proteins or other glutathione-dependent proteins can be purified directly from bacterial lysates using this bead with very high purity. Also the mild non-denaturing elution condition preserves protein antigenicity.

### TECHNICAL SPECIFICATIONS

BEAD GEOMETRY & SIZE	: Spherical, ~ 50 - 150 µm diameter
CROSSLINKED	: Yes
BEAD AGAROSE %	: 4%
ACTIVATING GROUP	: Epoxy
SPACER ARM	: 12 atom spacer arm (10 carbons)
PURIFICATION CAPACITY	: ~30 mg GST tagged fusion protein/ml pack bead
MATRIX STABILITY	: Stable in all commonly used reagents
STORAGE SOLUTION	: 20% aqueous ethanol
STORAGE TEMPERATURE	: 4°C to 8°C. <b>DO NOT FREEZE.</b>

M C sup FT W E1 E2 E3 E4 E5



SDS gel (12%) profile of purification of His-GST protein from crude bacterial cell lysate using Glutathione Agarose Beads. M:marker; C:crude; FT:flow through; W:wash; E1,2,3,4,5: elution1,2,3,4,5



## Affinity purification using Glutathione Agarose beads

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Carry all procedure at 4°C, unless specified (cold room). Bacterial cell pellet containing the desired GST tagged fusion protein was resuspended in 0.1M Phosphate buffer, pH 7.3 containing 0.15M NaCl and lysate was prepared by sonication/French press or any other method. Centrifuge the lysate at 13,500 rpm for 30 mins at 4°C to pellet the cellular debris. Collect the clear supernatant for purification process.

- ❖ Wash the bead with sufficient water to remove the preservative solution completely (select the bead volume depending on the approximate protein amount to be purified).
- ❖ Wash the bead with desired buffer (generally 0.1M Phosphate buffers, pH 7.3 containing 0.15M NaCl) and equilibrate in it for 15-30 mins.
- ❖ Incubate the protein mixture or bacterial lysate containing the desired GST tagged fusion protein to be purified with the bead for 2 hrs in 4°C, under gentle shaking.

*[For better purification carry the binding in dilute condition]*

- ❖ Load mixture in gravity flow column and collect the pass through as unbound fraction (use BioBharati Empty Gravity Column BB-EGC06/012/030).
- ❖ Wash the bead with approx 100-150 bead volume of phosphate buffer (PBS) at 4°C with occasional resuspension of the bead.

*[Proper wash is required to get eluted protein of over 90% purity]*

- ❖ Make the elution with 0.1M phosphate buffered saline pH 7.3 containing 10 mM reduced glutathione (concentration may be increased if required).

*[Make the elution stepwise in small fractions every time. Resuspend beads every time after addition of elution buffer and allow for few minutes]*

- ❖ Choose right fraction containing high amount of desired GST tagged fusion protein by protein amount estimation and SDS gel.
- ❖ Concentrate the fractions, dialyze it in desired buffer and store in -20°C /4°C according to its temp sensitivity.

For long term use and to keep the purification performance of bead good, it is important to wash the bead properly every time after purification by flushing plenty of double distilled water thoroughly with repeated resuspension and store it in 20% aqueous ethanol at 4°C.

[For anymore technical assistance please contact at [contact@nextgenbioproducts.com](mailto:contact@nextgenbioproducts.com)]