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ProductInformation

Glutathione Magnetic Agarose Beads

Product Code **G 1919**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Glutathione Magnetic Agarose Beads consist of a paramagnetic, immobilized metal-ion affinity chromatography (IMAC) resin, designed for use in automated and small-scale affinity capture (molecular pull-down) purifications. The Glutathione Magnetic Agarose Beads are designed to capture proteins with glutathione binding sequences, such as native glutathione S-transferase (GST), glutathione peroxidase, and glyoxalase I, while exhibiting low non-specific binding of other proteins. The glutathione ligand is covalently attached through the sulfur to epoxy-activated 6% magnetic agarose beads, resulting in a neutral 12 atom spacer.

This resin is designed to capture glutathione-binding target proteins from cell lysates and other biological samples in a manner similar to the standard Glutathione Agarose (Product Code G 4510). The magnetic properties of the resin allow for separation of the beads with the bound protein from the surrounding solution with use of a magnet. These properties of the resin allow for very rapid processing and aid in manipulations, such as repetitive washings, and recovery of the protein bound beads. This leads to greater speed, experimental reproducibility, and more accurate quantitation of the proteins of interest.

The capacity of these magnetic beads is greater than 15 mg of protein per ml of packed resin as determined with a glutathione binding protein (approximately 30 kDa). The matrix of the magnetic beads is a 6% beaded agarose with an average diameter of 50 µm and a diameter range of 20-75 µm. Paramagnetic iron is impregnated within the beads.

The Glutathione Magnetic Agarose Beads are supplied as a 50% slurry suspension in a 30% ethanol solution (antimicrobial preservative).

Reagents and Equipment Required But Not Provided

- TBS - 50 mM Tris Buffered Saline (138 mM NaCl and 2.7 mM KCl), pH 8.0 (Product Code T 6664)
- Reduced Glutathione (Product Code G 4251)
- Magnetic Separator:

For Microcentrifuge Tubes	M 1167
For Tissue Culture Flasks	M 1292
For Centrifuge Tubes	M 1542

Precautions and Disclaimer

The Glutathione Magnetic Agarose Beads are for laboratory use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

It is recommended to store the Glutathione Magnetic Agarose Beads as supplied at 2–8 °C. The unopened product is stable for at least two years. The resin should be stored in a solution containing 30% ethanol and kept at 2–8 °C for maximum stability and prevention of microbial growth.

Preparation Instructions

Glutathione Magnetic Agarose Beads are provided in a 30% ethanol solution. Thoroughly resuspend the affinity resin with gentle inversion and remove an appropriate aliquot for use. Take only the amount of gel suspension that is necessary for the purification to be done.

For affinity capture reactions, it is recommended to use 20 µl of the gel suspension per well (10 µl of packed gel) for automated 96 well purification procedures. For pull-down purification, 100 µl of the gel suspension per reaction (50 µl of packed gel) is recommended. The amount of resin can be varied, depending on the amount of target protein in the sample and the type of magnetic separator utilized (see Table 1 for approximate binding capacities).

Table 1.
Binding Capacity of Gel Suspension

Volume of Gel Suspension (µl)	Approximate Binding Capacity
10	75 µg
20	150 µg
50	375 µg
100	750 µg
200	1.5 mg

Equilibration and Wash Buffer - 50 mM Tris Buffered Saline (138 mM NaCl and 2.7 mM KCl), pH 8.0 (TBS, Product Code T 6664)

Elution Buffer - TBS, pH 8.0, with 10 mM reduced glutathione (Product Code G 4251)

Procedure
Note: It is recommended to read the entire technical bulletin before use, especially the Reagent Compatibility Chart (see Table 2).

Protein Preparation
The protein sample preparation steps should be empirically determined by the end user. Prior to application to the affinity gel, the protein sample must be clarified by centrifugation or filtration. For optimal results, the pH of the sample buffer must be between pH 7.0 and 8.0. Consult the reagent compatibility chart for the use of other reagents.

A comparable volume of sample without glutathione binding protein may be used as a negative control to monitor non-specific binding.

Affinity capture of glutathione binding proteins
There are many different procedures for performing small-scale affinity capture experiments. The procedure below is written for a single sample and is appropriate for most cell lines. The exact method used should be determined and optimized by the investigator, depending on the source of the sample (bacteria, fungi, plant cells, or tissue type). Additional information and procedures have been published.[1]

1. Carefully mix the Glutathione Magnetic Agarose Beads until uniformly suspended. Immediately transfer the appropriate amount of magnetic beads to a tube or well. To dispense the beads, use a wide orifice pipette tip. Wash the magnetic beads with 5 column volumes of Equilibration Buffer. Remove most of the Equilibration Buffer from the beads before use.
   Note: If the volume of the clarified crude extract is considerably larger (10x or more) than the volume of beads used, equilibration is not necessary.

2. Add the clarified crude extract containing the target protein. Gently mix the material on a plate shaker or an orbital shaker (approximately 175 rpm) for 30 minutes. Avoid magnetic stir bars and stirrers as the beads will bind to them.

3. Place the plate or tube in the magnetic separator for 10 seconds. Remove the solution and save for further analyses (SDS-PAGE). This solution contains the majority of the unbound protein.

4. Add up to 10 gel volumes of Wash Buffer to the affinity resin.

5. Mix the affinity resin suspension on a plate shaker or orbital shaker (approximately 175 rpm) for 1 minute. Use the magnetic separator and remove the Wash Buffer.

6. Repeat steps 3 and 4 to wash the affinity resin again. If desired, the affinity resin can be washed further with Wash Buffer until the A_{280} of the Wash Buffer no longer decreases. Discard the washes.

7. Add 5 gel volumes of Elution Buffer. Mix the affinity resin on a plate shaker or an orbital shaker (approximately 175 rpm) for 15 minutes.

8. Place the plate or tube in the magnetic separator for 10 seconds. Remove the eluate and save for further analyses. The target protein will be in this fraction.

Table 2.
Reagent Compatibility Chart

Reagent	Comments
Buffers	Tris or phosphate buffers, pH 6.5 to 9.5, are compatible with affinity capture with Glutathione Magnetic Agarose Beads.
Salts	Salt concentrations up to 1 M do not interfere with specific binding to the Glutathione Magnetic Agarose Beads.
Protease Inhibitors	Protease inhibitors such as EDTA, PMSF, or other serine protease inhibitors do not interfere with binding.
Detergents	The binding of glutathione binding protein to Glutathione Magnetic Agarose Beads is unaffected by 1% TRITON™ X-100, 1% TWEEN® 20, 1% CTAB, or 0.03% SDS.
Reducing Agents	The binding of glutathione binding protein to Glutathione Magnetic Agarose Beads is unaffected by 10 mM DTT.

References

1. Simons, P.C., and Vander Jagt, D.L., Purification of glutathione S-transferase from human liver by glutathione-affinity chromatography. Analytical Biochem., **82**, 334-341 (1977).
2. Simons, P.C., and Vander Jagt, D.L., Purification of glutathione S-transferases by glutathione-affinity chromotography. Methods Enzymol., **77**, 235-237 (1981).
3. Toribio, F., et al., Methods for purification of glutathione peroxidase and related enzymes. J. Chromatography, **684**, 77-97 (1996).

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