Technical Bulletin

Glutathione High Capacity Magnetic Agarose Beads

1:1 suspension in a 30% ethanol solution

G0924

Product Description

Glutathione High Capacity Magnetic Agarose Beads consist of a paramagnetic, immobilized metal-ion affinity chromatography (IMAC) resin with glutathione attached to it. Designed for use in automated and small-scale affinity capture (molecular pull-down) purifications, these beads are intended to capture proteins with glutathione-binding sequences, while exhibiting low non-specific binding of other proteins. Examples of native proteins include glutathione S-transferase (GST), glutathione peroxidase, and glyoxalase I.[1-3] The beads can also capture GST-tagged recombinant fusion proteins from cell lysates or other biochemical samples. These beads may be used for affinity capture, molecular pull-down, or immunoprecipitation (IP) of GST-tagged proteins.

The glutathione ligand is covalently attached via the sulfur to 4% paramagnetic agarose beads, which results in a neutral 12-atom (10-carbon) spacer. The magnetic properties of the resin allow for separation of beads with bound GST-tagged 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.

The matrix of the magnetic beads is a 4% beaded agarose with an average diameter of 50 µm and a diameter range of 20-75 µm. Paramagnetic iron is impregnated within the beads.

Several publications,[4-8] theses,[9] and dissertations[10-12] cite use of this G0924 product in their research protocols.

Reagent

The Glutathione High Capacity Magnetic Agarose Beads are supplied as a 50% slurry suspension (1:1 beads:solvent), where the solvent is a 30% ethanol solution.

Equipment Required but Not Provided

- TBS: 50 mM Tris-Buffered Saline (138 mM NaCl and 2.7 mM KCl), pH 8.0 (such as Cat. No. T6664)
- Reduced Glutathione (such as Cat. No. G4251)
- Magnetic Separator (such as Cat. No. M1167)

Do not use a magnetic stirring system. A magnetic stirring system will destroy the resin beads.

Storage/Stability

Storage at 2-8 °C of the Glutathione High Capacity Magnetic Agarose Beads is recommended, for maximum stability and prevention of microbial growth.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

- Thoroughly resuspend the affinity resin with gentle inversion. Remove an appropriate aliquot for use. Take only the necessary amount of gel suspension for the purification.
- 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.
- Equilibration and Wash Buffer: 50 mM Tris Buffered Saline (TBS; 138 mM NaCl and 2.7 mM KCl), pH 8.0
- Elution Buffer: TBS, pH 8.0, with 10 mM reduced glutathione

Procedure

Protein Preparation

- The end user should empirically determine the protein sample preparation steps.
- 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.

Reagent Compatibility Chart

Reagent	Comments
Buffers	Tris or phosphate buffers, pH 6.5 to 9.5, are compatible with affinity capture protocols.
Salts	Salt concentrations up to 1 M are not expected to interfere with specific binding.
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 is unaffected by 1% TRITON™ X-100, 1% TWEEN® 20, 1% CTAB, or 0.03% SDS.
Reducing Agents	The binding of glutathione-binding protein is unaffected by 10 mM DTT.

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

Many different procedures exist to perform small-scale affinity capture experiments. The following procedure is written for a single sample and is appropriate for most cell lines. The individual investigator should determine and optimize the exact method, depending on such factors as the sample source (bacteria, fungi, plant cells, or tissue type).

1. Carefully mix the Glutathione High Capacity 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 (10× or more) than the volume of beads used, then 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 (~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. Save the solution for further analyses (such as by 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 (~175 rpm) for 1 minute. Use the magnetic separator. 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 (~175 rpm) for 15 minutes.
8. Place the plate or tube in the magnetic separator for 10 seconds. Remove the eluate. Save the eluate for further analyses. The target protein will be in this fraction.

References

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