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Glutathione Agarose

1 Contents

Cat. No.	HY-K0211 -5 mL	HY-K0211 -10 mL	HY-K0211 -50 mL
Glutathione Agarose (Settled Resin)	5 mL	10 mL	50 mL

2 General Information

MCE Glutathione Agarose, a 4% highly cross-linked agarose reagent, effectively purifies high levels of overexpressed GST-tagged fusion proteins at a variety of scales. MCE Glutathione Agarose has high protein-binding capacity and stability, and is compatible with a wide range of chemicals and pH values, making it ideal for high performance purification of GST-tagged fusion proteins expressed in E. coli, yeast, insect and mammalian expression systems.

3 Characteristics

Composition	4% highly cross-linked agarose
Diameter	45-165 μm
Binding Capacity	＞10 mg GST-tagged protein (40 kDa)/mL Settled Resin
pH stable range	3-12
Maximum Pressure	0.3 MPa, 3 bar
Storage Solution	50% slurry in a 20% ethanol solution

4 General Protocol

4.1 Recommended Buffer

Binding/Wash Buffer	140 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4; filtered and sterilized using 0.22 μm or 0.45 μm filter
Elution Buffer	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0; filtered and sterilized using 0.22 μm or 0.45 μm filter

Note: 1-10 mM DTT could be contained in Binding/Wash Buffer or Elution Buffer.

4.2 Load of Glutathione Agarose

(1) Mix the slurry by gently inverting the bottle several times to completely suspend the agarose.

(2) Transfer an appropriate-sized slurry to the column and allow the agarose to settle down and the storage buffer to drain from the column.

(3) Equilibrate the column with 3× bed volumes of ultrapure water.

(4) Close the bottom outlet of the column.

4.3 Procedure

(1) Equilibrate the column of agarose with 5× bed volumes of Binding/Wash Buffer.

(2) Apply appropriate-sized sample to the column. If the sample contains insoluble matter, centrifuge or filter (0.22 μm or 0.45 μm filter) before use.

Note: Binding capacity is flow rate-and protein-dependent. Higher flow rates will decrease production time, but may result in losing a small portion of the target protein.

(3) Collect and save the flow-through for analysis.

(4) Wash the column with approximately 10-15× bed volumes of Binding/Wash Buffer or until the absorbance is stable. If desired, save supernatant for downstream analysis.

(5) Elute with approximately 5-10× bed volumes of Elution Buffer, collect fractions.

(6) Analyze the target protein by SDS-PAGE, along with fractions collected from different steps if necessary.

4.4 Cleaning-in Place, CIP

(1) To remove precipitated or denatured proteins, wash the column with 2× bed volumes of 6 M guanidine hydrochloride followed by 5× bed volumes of PBS (pH 7.4) washed.

(2) To remove hydrophobic substances, wash the column with 3-4× bed volumes of 70% ethanol or 2× bed volumes of 1% Triton X-100 followed by 5× bed volumes of PBS (pH 7.4) washed.

(3) Store agarose in 20% ethanol at 2-8°C.

5 Storage

Store at 2-8ºC, and is stable for at least 2 years.

6 Precautions

(1) Do not dry or freeze the agarose beads.

(2) This product is for R&D use only, not for drug, house hold, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

7 Troubleshooting

Problem	Possible Cause	Solution
High background	Lysate was highly particulate	Centrifuge or filter (0.22 μm or 0.45 μm) before use
		Sonicate the solution on ice; add 10 μg/mL RNase A and 5 μg/mL DNase I and incubate on ice for 10-15 minutes
	Lysate was too viscous	Remove organic solvent or protein stabilizing reagent (glycerol); decrease flow rate
Low protein yield	Poor expression of protein	Optimize expression conditions
	Not enough sample was loaded	Reapply the flow-through to the column
	Denaturation of GST tagged protein	Use mild lysis conditions
	Over cleavage denaturation of target protein	
	Protein precipitation	Add DTT to the solution before cell lysis, the final concentration is 1-20 mM
	The fusion protein changed the conformation of GST and affected the binding capacity of the target protein	Determine the binding capacity of GST in vector pGEX
		Reduce the binding temperature to 4°C and clean it sufficiently
	Insufficient washing	Increase washing time
	Low glutathione concentration in Elution Buffer	Increase the reduced glutathione concentration to 20-40 mM
	low pH value in Elution Buffer	Increase the pH to 8.0-9.0
		Increase the ionic strength, such as 0.1-0.2 M NaCl
	Glutathione is oxidized in Elution Buffer	Use freshly prepared Elution Buffer
		Add DTT
Poor protein purity	Protein degradation	Perform all procedure steps on 4ºC Add some protease inhibitor Use protease-deficient host bacteria
	Excessive cell disruption	Reduce cell disruption time
		Add lysozyme before ultrasound
	Column was too dirty	Perform the "Clean-in Place"