

PROCEDURE FOR USE GLUTATHIONE AGAROSE BEADS Cartridges (1 ml)

DESCRIPTION

Glutathione Agarose Resin is used to purify recombinant derivatives of glutathione S-transferases or glutathione binding proteins. Cartridges are “ready to use” FPLC™ columns prepacked with Glutathione Agarose Resin for rapid purification of GST fusion proteins. This cartridge can be used with an automated chromatography system, a peristaltic pump or with a syringe for manual processing.

This product is supplied in 20% ethanol.

INSTRUCTIONS

Cartridges (1 ml) can be operated with liquid chromatography systems (such as ÄKTA™ design systems) via standard 10-32 fittings without additional connectors.

1. Connect cartridge to the chromatography system

Purge the pump with binding buffer. Assure that all air is displaced. Remove the snap-off end at the column outlet and save it for further use.

Remove the upper plug from the column.

Fill the inlet port of the column with several drops of PBS to remove air to form a positive meniscus.

Start the pump at a flow rate of approximately 0.3 ml /min.

Insert the fitting “drop-to-drop” into the column port to avoid introducing air bubbles.

Note: Binding buffer: 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 2.7 mM KCl, 140 mM NaCl, pH 7.3 (PBS buffer). Prepare and filter (0,45 µm filter) before use.

Note: The snap-off end can be reused as a stop plug for a sealing the column outlet for storage.

2. Equilibration of the cartridge

Equilibrate the cartridge with 5- 10 ml of PBS buffer until the baseline at 280 nm is stable.

Note: A pre-equilibrated Glutathione Agarose cartridge may be used directly or may be stored at 4°C for up to 1 month and used if required.

3. Application of the Sample

Once the cartridge is equilibrated, the sample is applied. Load the centrifuged or filtered sample onto the column. Binding between GST and Glutathione agarose is relatively slow. A slight increase of contact time may facilitate binding. Therefore use low flow rates (0.1 – 0.3 ml/ min) for the loading step to allow maximal binding of the target protein.

Note: The binding capacity of the cartridge is 10 mg GST tagged protein. This is an orientative value for binding capacity. Binding capacity will vary for each GST-tagged protein.

The yield of GST-tagged protein depends on various parameters, such as nature of the fusion protein, expression host, culture conditions, etc. However, some recommendations on protein load and culture size can be given. Culture volume requirements are based on the followings assumptions:

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Recommendations

Protein load and culture size

The expression level of GST-tagged proteins is high ranging from 10^(a) to 50^(b) mg/liter of *E. coli* culture.

Table 1: Required culture volumes for Glutathione Agarose cartridges.

Expression level	<i>E. coli</i> culture	Resuspend in	Protein lysate
10 mg / l ^(a)	800 ml culture (~ 3.2 g cell pellet ⁽¹⁾)	~ 16 ml PBS ⁽²⁾	~ 20 ml
50 mg / l ^(b)	160 ml culture (~ 0.64 g cell pellet ⁽¹⁾)	~ 3.2 ml PBS ⁽²⁾	~ 4 ml

⁽¹⁾. On average, 250 ml of culture will produce approximately 1g of pelleted, wet cells.

⁽²⁾ 1g cells may be lysed in 2-5 ml PBS.

4. Washing of the Cartridge

Wash the cartridge (flow rate 1 ml /min) with 10 ml of binding buffer until the O.D. 280 nm is stable.

5. Elution of the Pure protein

Elute the target protein with 10 ml of Elution buffer and collect the purified protein.

Note: Elution buffer: 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0. It is possible that a significant amount of GST fusion protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST fusion proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-fused protein).

6. Regeneration & Storage

During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary.

In general, cartridge regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do regeneration when an appreciable diminution in the yield is observed. The frequency of these stages varies with the protein and the conditions used.

After use, regenerate the resin by sequentially applying 10 ml of 100 mM Tris-HCl, 0.5 M NaCl, pH 8.5 followed by a second step with 10 ml of 100 mM sodium acetate, 0.5 M NaCl pH 4.5. Repeat the above wash cycles twice and finally wash with 5 ml of binding buffer. If you will not be using the resin immediately wash with 5 ml of 20% ethanol and store at 4°C.

For laboratory use only. Not for use in diagnostic or therapeutic procedures.

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