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GSTPur Glutathione kit

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1. Product Description

Glutathione Beads is an affinity chromatography medium designed for one-step purification of recombinant glutathione S-transferase (GST) fusion proteins and other glutathione binding proteins expressed in *E. coli*, insect cells and mammalian cells. The recombinant GST fusion proteins can be purified directly from pre-treated cell lysate using Glutathione Beads. It is the excellent choice for high performance purifications. **GSTPur Glutathione kit** contains one GSTPur Glutathione Column, Lysis Buffer and Elution Buffer. It is fast, simple and easy operation.

Table 1. Characteristics of **Glutathione Beads**

Item	Description
Matrix Spherical	4% agarose
Static Binding Capacity	>20mg GST-tagged protein(40kDa)/ml medium
Particle size	45-165um
Maximum Pressure	0.1MPa, 1bar
Storage Solution	1X PBS containing 20% ethanol
Storage Temperature	2-8℃

Table 2. The component of **GSTPur Glutathione Kit**

	Cat.No	Size	Amount
GSTPur Glutathione Column	SA008K-1	3ml	1
Lysis Buffer	SA008K-2	200ml	2
Elution Buffer	SA008K-3	200ml	1

2. Purification Procedure

2.1 Sample Preparation

It is recommended to filter the sample solution by passing them through a 0.22μm or 0.45 μm filter before use.

2.2 Column Purification

GSTPur Glutathione kit contains GSTPur Glutathione Column, Lysis Buffer and Elution Buffer. The purification process takes about 30min. It mainly depends on the sample volume and solution viscosity.

1) Fix GSTPur Glutathione Column and remove the bottom plug and top plug. Allow storage buffer to drain from resin by gravity flow.

2) Equilibrate column with 5ml Lysis Buffer. Allow buffer to drain from the column. Repeat this step two times.

3) Add the prepared protein extract to the resin. Collect the flow-through. If desired, re-apply the flow-through once to maximize binding.

4) Wash resin with 5ml Lysis Buffer and collect the flow-through. Repeat this step five times or repeat this step using a new collection tube until the absorbance of the flow-through fraction at 280nm approaches baseline.

5) Elute GST-tagged proteins from the resin with 15-30ml Elution Buffer. Collect the fraction every 5ml in a separate tube.

6) Equilibrate the column with 5ml Lysis Buffer and 5ml deionized water alternately. Repeat 2 times. Then Equilibrate the column with 5ml20% ethanol. Finally store the beads with 1XPBS containing 20% ethanol at 2-8℃.

2.4 Analysis

IIdentify the fractions containing the protein. Use UV absorbance, SDS-PAGE, or western blot.

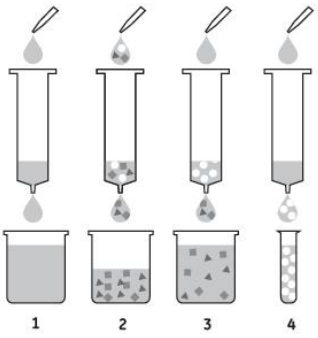

Fig.2. Purification process of GSTPur Glutathione Column

Step1：Equilibrate；Step2：binding；Step3：Wash；Step4：Elution

3. Cleaning-in-Place

GSTPur Glutathione Column can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, the Glutathione resin must be regenerated using the following protocol:

Remove the precipitation or denatured protein

Wash the column with 2 column volumes 6M guanidine hydrochloride solution. Finally wash the column with 5 column volumes 1XPBS (pH7.4).

Remove the hydrophobically bound protein

Wash the column with 3-4 column volumes 70% ethanol or 2 column volumes 1% Triton X-100. Finally wash the column with 5 column volumes 1XPBS (pH7.4).

4. Troubleshooting

Problem	Probable Cause	Solution
The yield of the purified GST fusion protein is low or undetectable.	The fusion protein forms inclusion body.	Grow bacteria at lower temperature (20-30°C), or reduce final concentration of IPTG to 0.1 mM for protein induction, or reduce the induction time.
		Properly dissolve and refold the inclusion body prior to the purification.
	The fusion protein does not bind to Glutathione Beads efficiently.	Use batch method for purification. Incubate clear solution (the sonicate, etc) containing GST-fusion protein with Glutathione Beads for 2 hours or longer (such as overnight) and then load the mixture onto the column.
	The fusion protein does not contain active GST.	Use mild sonication condition or other lysis method, such as lysozyme so that GST is not denatured.
	The fusion protein is degraded by protease.	Add appropriate protease inhibitors such as PMSF in the lysis solution and wash solution.
	The fusion protein is not efficiently eluted from Glutathione Beads.	Increase elution time or increase the concentration of glutathione to 15 mM or higher in the elution buffer.
		Adjust the pH of the elution buffer to 8.0-9.0 without increasing the glutathione concentration.
		Add Triton X-100 (0.1%, final concentration) or Noctylglucoside (2%, final concentration) or NaCl (0.1-0.2 M, final concentration) to the elution buffer.
Multiple bands observed in the eluted protein	The fusion protein is degradated by protease.	Add appropriate protease inhibitors (or inhibitor cocktails) such as PMSF in the lysis solution and wash solution.
	Some host proteins,such as chaperonins, may interact with the fusion protein.	Add DTT (5 mM, final concentration) in the wash buffer. Incubate the recombinant protein solution in chaperonin buffer (2 mM ATP, 10 mM MgSO4, 50 mM Tris-HCl) at 37°C for 10 min prior to the purification.
	Over-sonication will cause some protein to bind to the fusion protein.	Use milder sonication condition or another lysis method.
	Some protein will bind to the fusion protein or beads non-specifically.	Optimizing the wash conditions. Detergents such as 1% Triton X-100, 1% Tween-20, 0.03% SDS, or 0.1% NP-40 may be used to reduce non-specific binding. Salt concentration in the wash solution can also be optimized to reduce non-specific binding.

5. Related Products

Product	Cat. No.	Size
Glutathione Beads	SA008005	5 ml
	SA008025	25 ml
	SA008100	100 ml
	SA008500	500 ml
	SA00801L	1 L
	SA00810L	10 L
GSTPur Glutathione Kit	SA008K03	3 次
	SA008K05	5 次
Glutathione Beads 4FF	SA010005	5 ml
	SA010025	25 ml
	SA010100	100 ml
	SA010500	500 ml
	SA01001L	1 L
	SA01010L	10 L
GSTCap 4FF	SA010C11	1X1 ml
	SA010C51	5x1 ml
	SA010C15	1X5 ml
	SA010C55	5X5 ml
	SA010CS	3X1 ml+1X5 ml