Increasing the yield of purified GST-tagged proteins

After polyhistidine, GST is the most used tag in protein purification. The GST tag has many advantages, including high specificity, increased target protein solubility, and mild elution conditions, which preserve the activity of the protein. Although the GST tag has been used successfully for decades, unsatisfactory yields have been reported for low-expressed and/or high molecular weight proteins. To evaluate how these parameters affect yield, fusion proteins with different molecular weight at various concentrations were purified.

The purification performance of various GST-tagged proteins was compared using two chromatography media with immobilized glutathione ligands: Glutathione Sepharose™ 4B, a medium with high binding capacity, and Glutathione Sepharose 4 Fast Flow, which is designed for higher flow rates. Prepacked columns, GSTrap™ 4B and GSTrap FF, were used and the experiments were carried out with low or high load of GST-tagged protein. Different sample concentrations were achieved by spiking purified GST-tagged proteins into untransformed *E. coli* lysates.

All purifications were performed using the following protocol:

Columns:	GSTrap 4B and GSTrap FF, 1 ml
Binding/wash buffer:	10 mM sodium phosphate, 140 mM sodium chloride, 2.7 mM potassium chloride, pH 7.4
Elution buffer:	50 mM Tris-HCl, 10 mM glutathione, pH 8
Flow rates:	Binding, 0.3 ml/min; Equilibration, wash, and elution, 1 ml/min
System:	ÄKTAexplorer™ 10
Detection:	Absorbance at 280 nm

Molecular weight and concentration affect yield of GST-tagged protein

The GST tag with the fusion protein forms dimers under normal purification conditions as observed in this series of experiments. The obtained yields varied significantly between the fusion proteins (Fig 1). Purification using GSTrap 4B gave yields above 50% for all proteins, and lower concentrations were observed to give lower yields (Fig 1 A and C). With GSTrap FF, yield was lower for high molecular weight proteins (Fig 1B and D). Lower yields at lower concentrations were also noted in this case.

Bacterial glutathione explains lower binding effects

A plausible explanation of the slightly lower binding at lower concentrations is that endogenous glutathione competes for binding sites. The concentration of glutathione in bacteria ranges from 0.1 to 10 mmol. To confirm the presence of endogenous glutathione in the lysates, a glutathione detection experiment was performed on cell lysates without any glutathione added. GST was detected by its enzymatic activity of conjugating CDNB and glutathione, yielding a yellow product that was detected at 340 nm (Fig 2). A sample containing GST (2 mg/ml) and reduced glutathione (10 mM) was used as standard. The experiments were performed in duplicates or triplicates, and the standard deviation is shown in Figure 2.

Low flow rates improve yield

The binding kinetics of GST to glutathione was slow, K_m 0.43 ±0.07 mM, and therefore, maintaining a low flow rate (approx. 0.3 ml/min) during sample application was necessary to obtain good yields.

Recommendations

- Use Glutathione Sepharose 4B for high molecular weight proteins
- If the protein is poorly expressed, endogenous glutathione can interfere
- For good protein recoveries, maintain a low flow rate (approx. 0.3 ml/min) during sample application

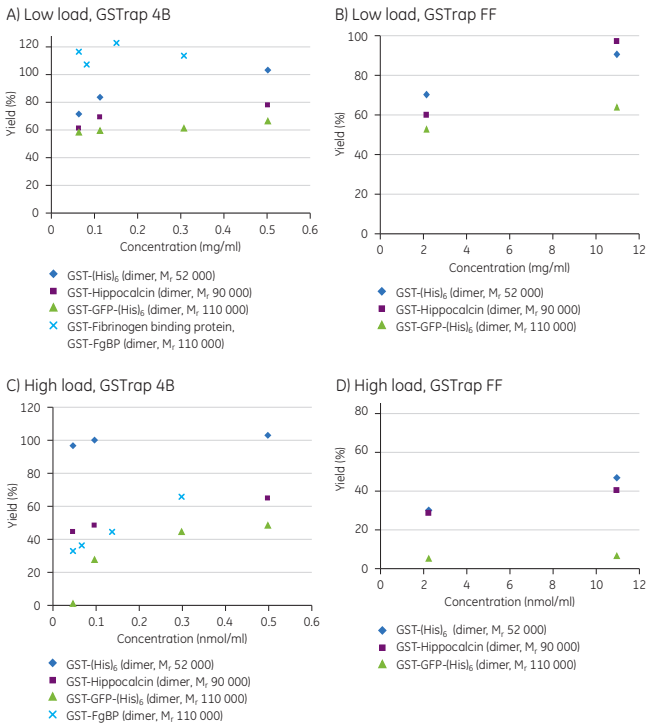

Fig 1. Yields from purifications using GSTrap 4B and GSTrap FF with varying concentration of target protein in starting material at **A)** and **B)** low load (0.5 and 1 mg) and **C)** and **D)** high load (5.8, 10, and 12 mg), respectively. Note that the M_r 26 000 GST protein forms dimeric complexes under normal purifications conditions, as observed in this study. The molecular weights for GST dimer complexes are given in the Figure.

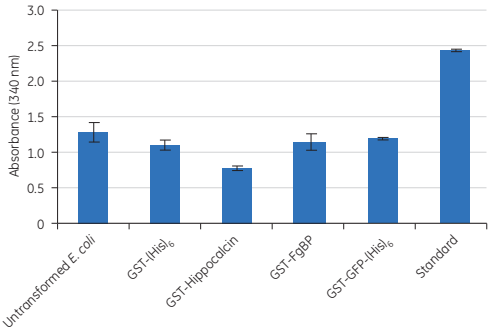

Fig 2. Detection of endogenous glutathione in cell lysates.

Ordering information

Product	Code number
GSTrap 4B, 5 × 1 ml	28-4017-45
GSTrap 4B, 5 × 5 ml	28-4017-48
GSTrap FF, 5 × 1 ml	17-5131-01
GSTrap FF, 5 × 5 ml	17-5131-02

For more information on purification products for GST-tagged proteins, visit www.gelifesciences.com/protein-purification