

GST•Bind™ Chromatography

GST•Bind™ Fractogel® columns take advantage of the binding interaction between the glutathione-S-transferase protein (such as GST•Tag™ sequence, 220 amino acids, M_r 26 kDa) and glutathione (307.2 Da). Due to the high specificity of correctly folded GST for glutathione, target proteins can be quickly and easily purified to near homogeneity in a single step (Figure 2). Applying the sample using a slow flow rate (ideally 0.1-0.5 ml/min) maximizes binding (Figure 3). Target proteins are eluted from the resin using very mild conditions (10 mM reduced glutathione), which helps preserve protein activity.

The GST•Bind Buffer Kit includes an optimized Bind/Wash Buffer and Glutathione, Reduced for use with GST•Bind Fractogel Cartridges. Sample preparation buffers and the Bind/Wash Buffer can be supplemented with a wide variety of additives, including 2-mercaptoethanol, dithiothreitol, EDTA, and protease inhibitor cocktails. Cell lysates can be prepared using traditional mechanical lysis methods or by using convenient chemical lysis reagents, such as BugBuster® Protein Extraction Reagent (Figures 1-3). BugBuster Master Mix (premixed Benzonase® Nuclease and rLysozyme™ Solution), and PopCulture® Reagent.

Conclusion

GST•Bind Fractogel Cartridges provide fast, efficient single-step purification of GST fusion proteins, either manually or with an automated liquid chromatography system. The stable Fractogel-based resin can be regenerated and reused for at least 10 reproducible, high-yield purifications. Also available are BugBuster Protein Extraction Reagent, BugBuster Master Mix, PopCulture Reagent, Benzonase Nuclease, rLysozyme Solution, and the GST•Bind Buffer Kit for fast, convenient, and reliable GST fusion protein purification. ■

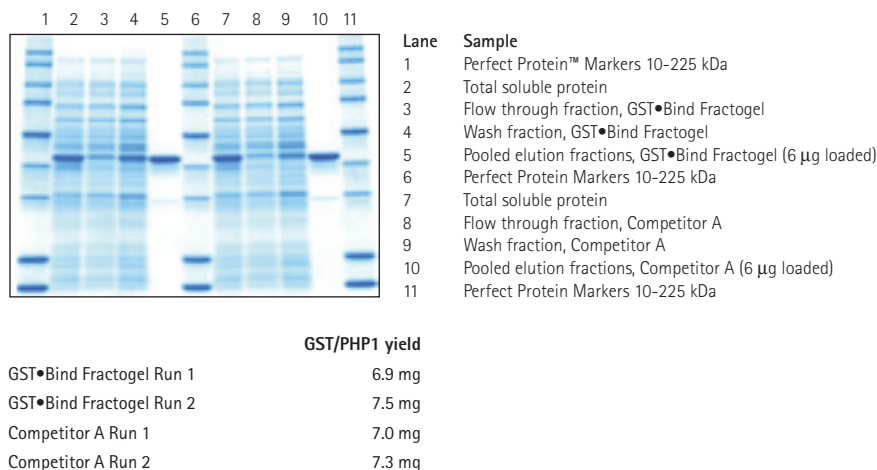


Figure 2. Comparison of GST•Tag fusion protein purification using a GST•Bind Fractogel Cartridge or another GST affinity column

A crude cell lysate containing a GST/PHPT1 fusion protein was prepared as described for Figure 1. The same soluble fraction was used for all purifications: two replicates each for a single GST•Bind Fractogel Cartridge or a single 1.0-ml GST affinity column from competitor A. Columns were re-equilibrated between runs by washing with 25 cv GST Bind/Wash Buffer. The buffer system, buffer volumes, and flow rates used were as described for Figure 1. Elution fractions containing GST/PHPT1 were identified by SDS-PAGE, pooled, and concentrations were determined by measuring A_{280} (extinction coefficient = 1.705 for 1.0 mg/ml solution). Gel shows results for Run 2, after re-equilibration. Run 1 results were comparable.

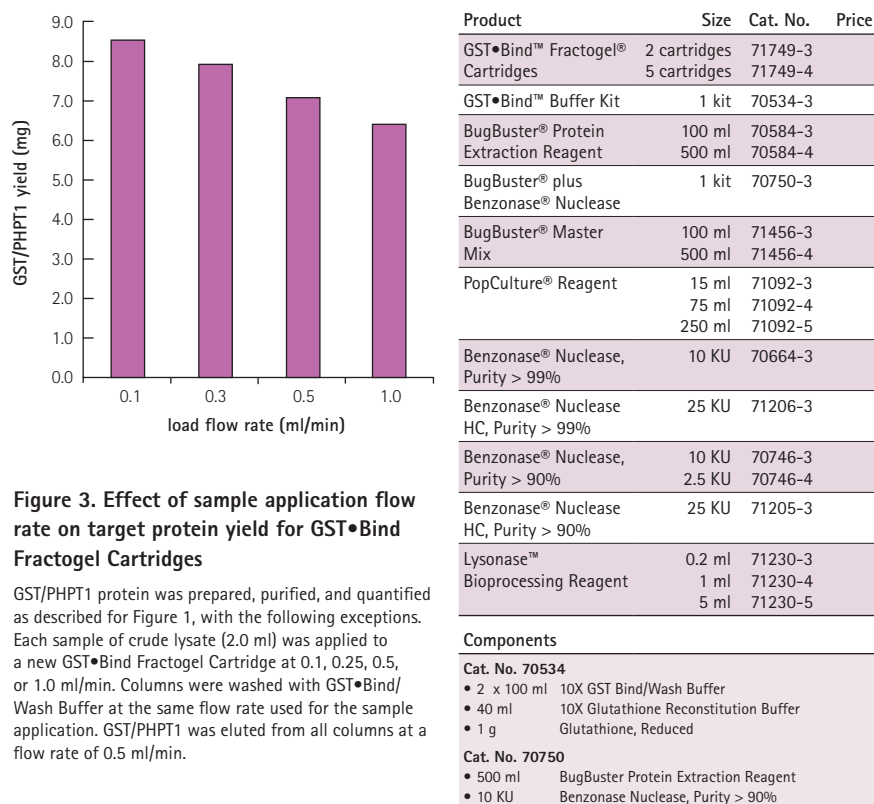


Figure 3. Effect of sample application flow rate on target protein yield for GST•Bind Fractogel Cartridges

GST/PHPT1 protein was prepared, purified, and quantified as described for Figure 1, with the following exceptions. Each sample of crude lysate (2.0 ml) was applied to a new GST•Bind Fractogel Cartridge at 0.1, 0.25, 0.5, or 1.0 ml/min. Columns were washed with GST•Bind/Wash Buffer at the same flow rate used for the sample application. GST/PHPT1 was eluted from all columns at a flow rate of 0.5 ml/min.