Separation of DnaK from pGEX-GST by ion exchange chromatography

DnaK (the 70 kDa protein produced by the gene *dnaK*) is a common contaminant of GST fusion proteins produced in *E. coli*, even after Glutathione Sepharose® affinity chromatography. DnaK can be removed from GST by ion exchange chromatography using a method similar to one used in the purification of DnaK (1). The following methods were developed using the strong ion exchangers HiTrap® Q (17-1153-01), and RESOURCE® Q (17-1177-01), as well as the weak ion exchanger DEAE Sepharose® Fast Flow (17-0709-01). The chromatography conditions are given in Table 1.

Table 1. Chromatography conditions for separation of DnaK from GST using HiTrap® Q, RESOURCE® Q and DEAE Sepharose® Fast Flow

Sample:	**HiTrap Q and RESOURCE Q:** Affinity purified GST [5 mg/ml in 50 mM Tris-HCl (pH 8.0) + 10 mM glutathione] **DEAE Sepharose Fast Flow:** Affinity purified GST (5 mg/ml in PBS + 10 mM glutathione) diluted five-fold with Buffer A
Sample volume:	1 ml
Bed volume:	1 ml
Buffer A:	50 mM Tris-HCl (pH 8.0), 5 mM βmercaptoethanol, 0.1 mM EDTA, 10% glycerol
Buffer B:	**HiTrap Q and RESOURCE Q:** Buffer A + 1 M NaCl **DEAE Sepharose Fast Flow:** Buffer A + 400 mM NaCl
Gradient:	Step; 0% B for 10 ml, 20% B for 10 ml, 100% B
Flow rate:	**HiTrap Q and RESOURCE Q:** 1 ml/min **DEAE Sepharose Fast Flow:** 0.5 ml/min
Fraction size:	**HiTrap Q and RESOURCE Q:** 1 ml **DEAE Sepharose Fast Flow:** 1.5 ml
Detection:	214 nm and conductivity meter
System:	FPLC® System with FPLCdirector®

Using these conditions for HiTrap Q and RESOURCE Q, the peak corresponding to GST eluted at approximately 14 ml for both columns. The GST peak is well-separated from the DnaK peak, which eluted at approximately 23 ml (Figures 1A and 1B). Similar results were obtained for DEAE Sepharose Fast Flow; GST eluted at 12 ml and DnaK at 22 ml (Figure 1C).

GST and DnaK peak identities were confirmed by Western blot analysis using the anti-GST antibody supplied in the GST Detection Module (27-4590-01) and an anti-DnaK antibody (2). No DnaK was detected in the GST fractions (Figure 2; data not shown for DEAE Sepharose Fast Flow).

Although this procedure was developed for pGEX-GST without a fusion partner, these procedures should provide good starting points for working with GST fusion proteins. The presence of a fusion partner may alter the conditions under which the GST fusion protein will bind to and elute from these ion exchange media. The method can be optimized for a particular fusion protein by using a different step gradient or by using a linear gradient. Provided that the fusion protein elutes under conditions different from those for DnaK, separation of the two proteins can be achieved.

References

1. Zylicz, M. and Georgopoulos, C., *J. Biol. Chem.* **259**, 8820 (1984).
2. StressGen Biotechnologies Corp., Victoria, B. C., Canada; phone, 604-744-2811; fax, 604-744-2877; e-mail, support@stressgen.com

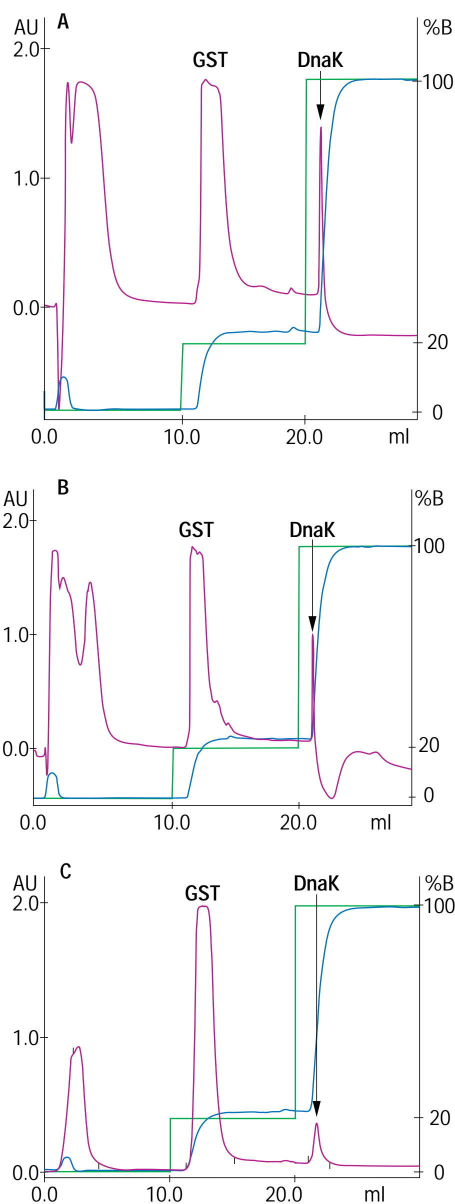

Figure 1. Separation of DnaK from GST by ion exchange chromatography. A) HiTrap® Q column; B) RESOURCE® Q column; C) DEAE Sepharose® Fast Flow HR 5/5. See Table 1 for details. Purple line = absorbance at 214 nm; blue line = conductivity; green line = theoretical gradient of Buffer B. Data courtesy of Tom Daniels and Jay Pink, Pharmacia Biotech, Milwaukee, WI, USA.

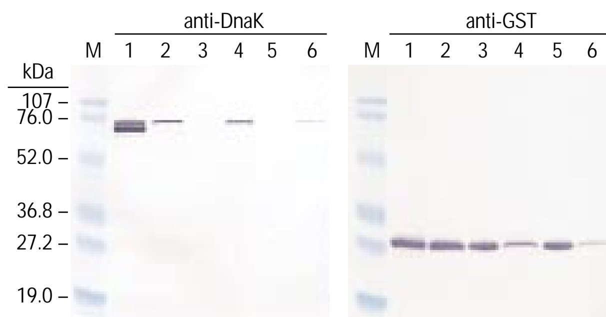

Figure 2. Western blot analysis of eluted peaks. Lanes 1, DnaK/GST standards (70 kDa and 27 kDa, respectively); lanes 2, column load material; lanes 3, GST peak from HiTrap® Q; lanes 4, DnaK peak from HiTrap Q; lanes 5, GST peak from RESOURCE® Q; lanes 6, DnaK peak from RESOURCE Q. M = molecular weight marker. Data courtesy of Tom Daniels, Pharmacia Biotech, Milwaukee, WI, USA.