Procedure

It is not unusual that, together with a protein overexpressed and purified from E.coli, to co-purify a certain chaperone. In such case, conventional separation protocols fail, because of the interaction between the chaperone and the protein. The method described below, based on Thain et al.(Trends in Genetics (1996) 12, 209-210), can be used to remove them in one step. The basic idea here is that ATP stimulates substrate release from GroEL; however, it has to borne in mind that ATP hydrolysis by GroEL occurs fast, leading to rebinding of GroEL to the proteic substrate; it is therefore necessary to wash the column as opposed to incubate it. [I used this protocol for GST columns; I don't know how it behaves on other, say His, columns, but I don't find any reason for which it would not work]

Bind the protein of interest to the affinity column and wash it thoroughly, as usual. Run a SDS gel in order to determine whether there are other proteins bound to the column, except the protein of interest. If yes,

Equilibrate the column in the HSP-removal buffer

Wash the column with HSP-removal buffer plus 5mM ATP (about 20 column volumes)

Recheck the matrix by SDS-PAGE. If the contaminant band is still there, you can try to wash the column with the HSP-removal buffer plus 5mM ATP supplemented with 2.5 ?M GroES (GroES stimulates substrate release by GroEL).

After the contaminant band has been removed, you can continue the purification protocol as usual.

Solutions

HSP-removal buffer

- 50mM Tris pH7.5
- 50mM KCl
- 20mM MgCl2

GroES can be purchased from Sigma