



# Automated multistep purification of (histidine)<sub>6</sub> – and GST-tagged proteins

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CY13484-15May20-PT

# Automated Multistep Purification of (histidine)<sub>6</sub>– and GST-tagged Proteins

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## Introduction

Automated multistep purifications of five affinity tagged proteins were performed using ÄKTAexpress™, a new chromatography system.

The following purification results are presented:

- One (histidine)<sub>6</sub>-tagged protein using four different multistep protocols
- Four different (histidine)<sub>6</sub>-tagged proteins using the same four-step protocol
- One (histidine)<sub>6</sub>-tagged and one Glutathione S-transferase (GST)-tagged protein including automatic tag removal

## ÄKTAexpress

- Automated multistep purification of affinity-tagged proteins
- Method wizard for easy creation of purification protocols
- Intelligent peak detection and collection in intermediate steps
- Optional on-column tag cleavage



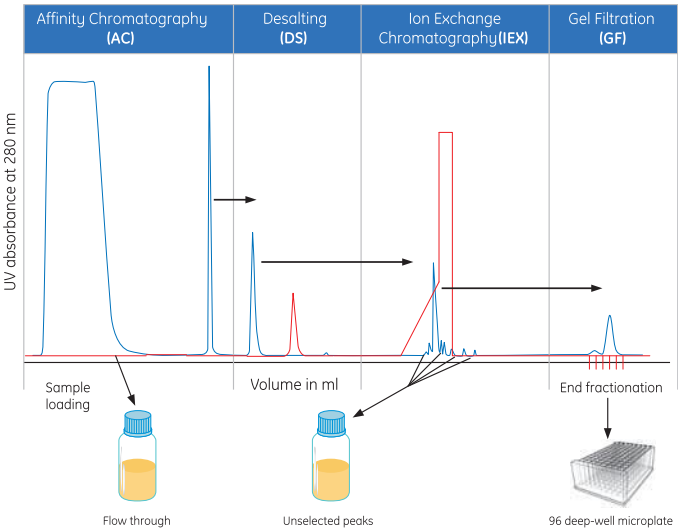
## Conclusions

- Automated multistep protocols were used to produce proteins with high purity.
- Different peak shapes in intermediate purification steps are automatically handled by the system.
- Automatic tag removal was successfully performed.
- Yields of up to 50 mg protein were obtained.



# Multistep purification using ÄKTAexpress

All protocols start with affinity chromatography followed by different combinations of desalting, ion exchange chromatography and gel filtration. The largest peak from each step is transferred to the next column.



## Available multistep protocols

AC-DS	Buffer exchange
AC-GF	Separation from undesired aggregates and contaminants
AC-DS-IEX	Separation from other isoforms (e.g. heterogenously phosphorylated or glycosylated proteins)
AC-DS-IEX-DS	Separation from other isoforms on IEX and buffer exchange on DS
AC-DS-IEX-GF	Separation from other isoforms on IEX and removal of undesired aggregates and contaminants on GF

## Effects from additional chromatographic steps

# Material and methods

## Samples

All used (histidine)<sub>6</sub>- and GST-tagged proteins were expressed in *E. coli*. After harvest, cell lysis was performed by sonication. The samples were clarified by centrifugation prior sample loading.

## Buffers

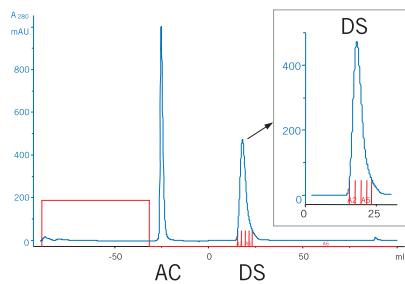
AC (His) binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5
AC (His) cleavage buffer:	50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5
AC (His) elution buffer:	50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.5
AC (GST) binding and cleavage buffer:	50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5
AC (GST) elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
DS and IEX binding buffer:	50 mM Tris-HCl, pH 8.0
IEX elution buffer:	50 mM Tris-HCl, 1 M NaCl, pH 8.0
GF buffer:	50 mM Tris-HCl, 150 mM NaCl, pH 7.5

## Analysis

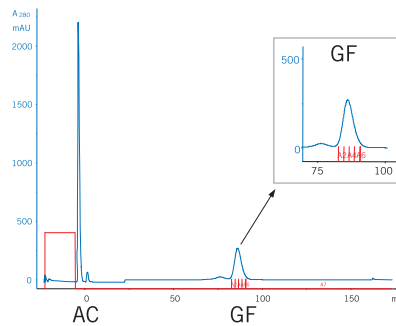
Purity of each sample was analyzed by Coomassie™-stained SDS polyacrylamide gels. The reduced samples were applied on 8–18 % gradient or 12.5 % homogenous ExcelGel™ SDS-PAGE gels. Approximately 7.5 µg of protein was loaded per lane.

## One protein and four different protocols

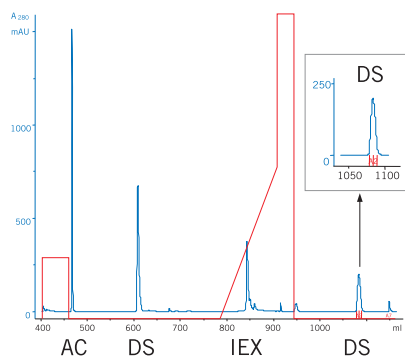
A (histidine)<sub>6</sub>-tagged protein, APB7 ( $M_r$  28×10<sup>3</sup>, pI 6.0), was purified using four different multistep protocols. The pooled fractions from the final purification step were analyzed by SDS-PAGE.



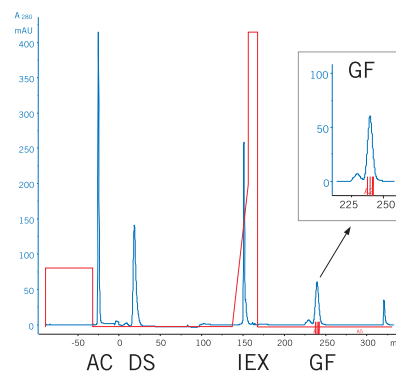
Columns:  
HisTrap™ HP, 5 ml;  
HiPrep™ 26/10 Desalting



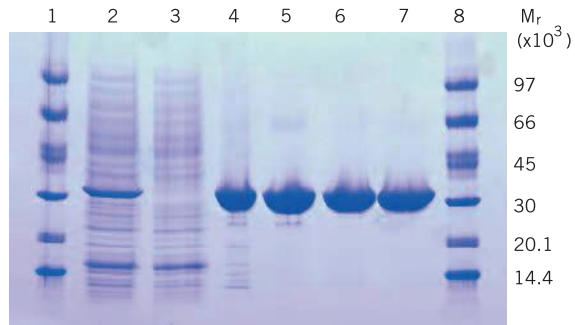
Columns:  
HisTrap HP, 1 ml;  
HiLoad™ 16/60 Superdex™ 75 pg



Columns:  
HisTrap HP, 5 ml;  
HiPrep 26/10 Desalting;  
RESOURCE™ Q, 6 ml



Columns:  
HisTrap HP, 5 ml;  
HiPrep 26/10 Desalting;  
RESOURCE Q, 1 ml;  
HiLoad 16/60 Superdex 75 pg



### SDS-PAGE analysis

The degree of purity as judged visually was in the following order:  
AC-DS < AC-GF < AC-DS-IEX-DS < AC-DS-IEX-GF.

- Lane 1. Low Molecular Weight marker
- Lane 2. Start sample
- Lane 3. Flow through
- Lane 4. AC-DS
- Lane 5. AC-GF
- Lane 6. AC-DS-IEX-DS
- Lane 7. AC-DS-IEX-GF
- Lane 8. Low Molecular Weight marker

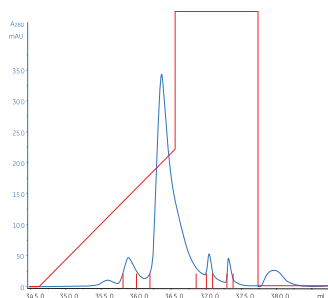
## Four different proteins and one protocol

Four (histidine)<sub>6</sub>-tagged proteins were purified using the same four-step protocol, AC-DS-IEX-GF. The ion exchange and gel filtration steps are shown below.

### Ion Exchange Chromatography

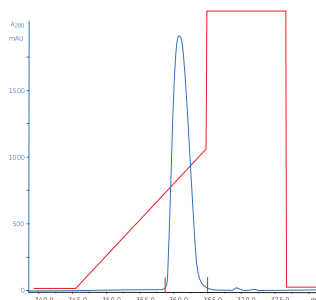
Sample:  
P-450,  $M_r$  121  $\times 10^3$ , pI 5.3

Column: HiLoad 16/60 Superdex 200 pg



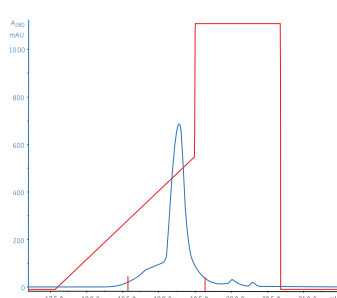
Sample:  
APC234,  $M_r$  32.5  $\times 10^3$ , pI 5.8

Column: RESOURCE Q, 1ml



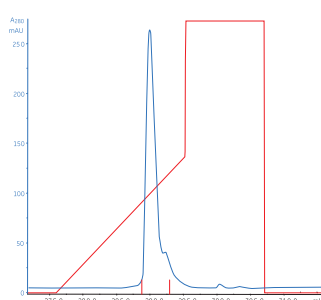
Sample:  
APC1040,  $M_r$  38.9  $\times 10^3$ , pI 5.7

Column: RESOURCE Q, 1ml



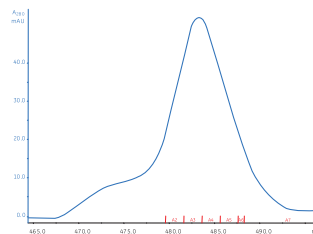
Sample: APB7,  
 $M_r$  28  $\times 10^3$ , pI 6.0

Column: RESOURCE Q, 1ml

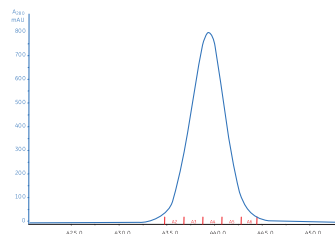


### Gel Filtration

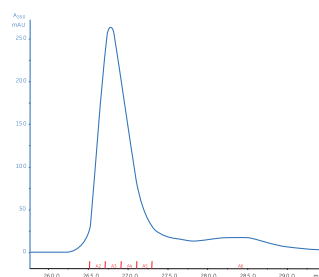
Column: RESOURCE Q, 1ml



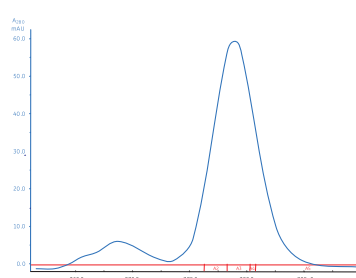
Column: HiLoad 16/60 Superdex 75 pg



Column: HiLoad 16/60 Superdex 75 pg



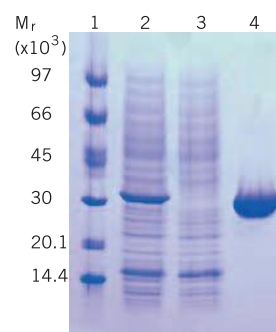
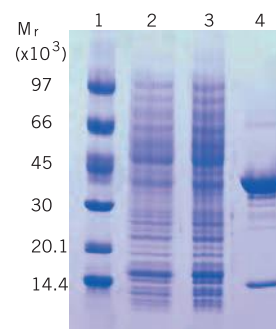
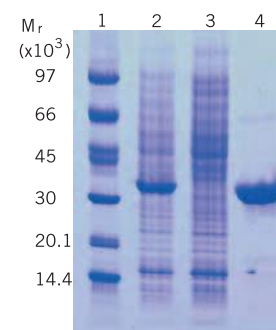
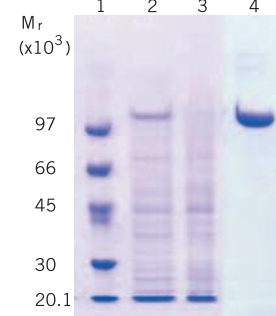
Column: HiLoad 16/60 Superdex 75 pg



### SDS-PAGE

Lane 1. Low Molecular Weight marker  
Lane 2. Start sample  
Lane 3. Flow through  
Lane 4. Pooled protein after AC-DS-IEX-GF

### SDS-PAGE



## Ion Exchange Chromatography

The software and hardware were able to detect and handle peaks with different shapes. The largest peak was injected on to the gel filtration column.

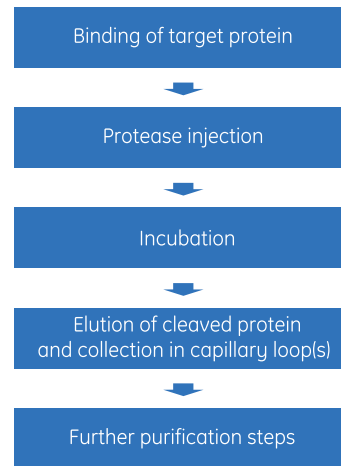
## Gel Filtration and SDS-PAGE analysis

In the final step, peak fractionation was performed and all peaks were collected in the integrated fraction collector. For all four proteins high purity was achieved using the same pre-optimized protocol.

## Automated tag removal

All multistep purification protocols in ÄKTAexpress can be combined with automated on-column tag cleavage. Tag cleavage is always performed on the affinity column prior further purification steps. When the cleaved protein has been eluted the affinity column is regenerated and affinity tag, tagged protease and remaining uncleaved protein is collected in separate outlet.

### Procedure

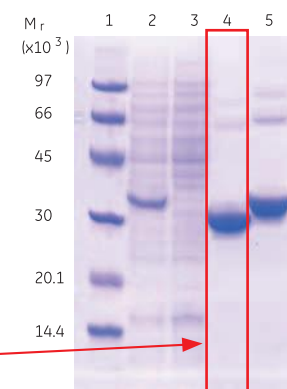
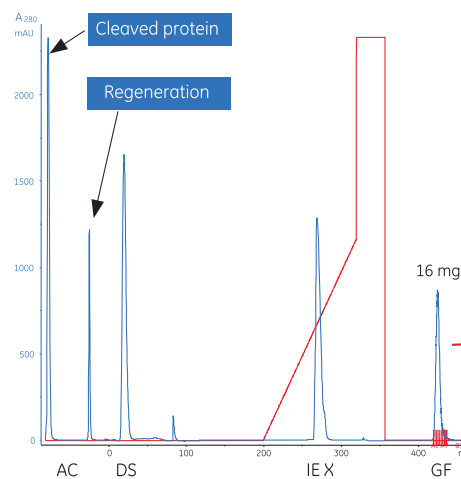


## Four-step protocol: (histidine)<sub>6</sub>-tagged protein cleaved with AcTEV protease

**Sample:** APC234,  $M_r$  32.5  $\times 10^3$   
(Cleaved product:  $M_r$  30.1  $\times 10^3$ )

**Columns:** HisTrap HP, 5 ml;  
HiPrep 26/10 Desalting;  
RESOURCE Q, 6 ml;  
HiLoad 16/60 Superdex 75 pg

**Cleavage conditions:** 200 units of AcTEV™ Protease (Invitrogen)/mg protein, 8 hours incubation time at room temperature.



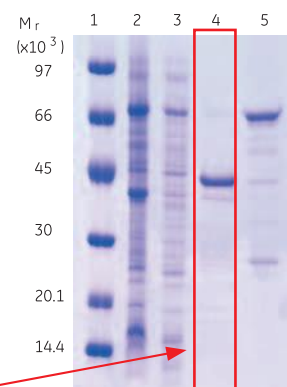
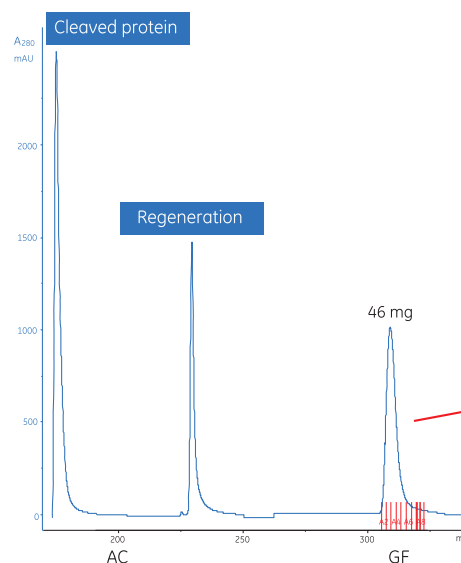
Lane 1. Low Molecular Weight marker  
Lane 2. Start sample  
Lane 3. Flow through  
Lane 4. Purified cleaved APC234  
Lane 5. Reference: uncleaved APC234

## Two-step protocol: GST-tagged protein cleaved with PreScission protease

**Sample:** GST-purα,  $M_r$  61.6  $\times 10^3$   
(Cleaved product:  $M_r$  35.2  $\times 10^3$ )

**Columns:** GSTrap™ HP, 5 ml;  
HiLoad 16/60 Superdex 75 pg

**Cleavage conditions:** 20 units of PreScission™ protease/mg protein, 8 hours incubation time in cold room.

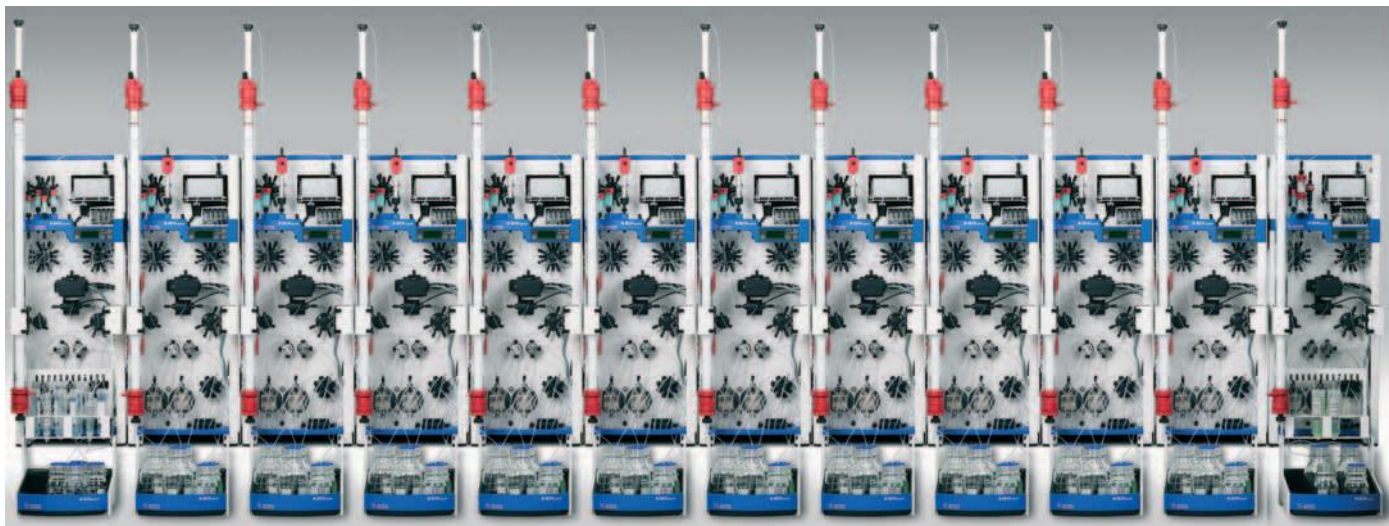


Lane 1. Low Molecular Weight marker  
Lane 2. Start sample  
Lane 3. Flow through  
Lane 4. Purified cleaved GST-purα  
Lane 5. Reference: uncleaved GST-purα



## High-throughput protein purification

- Up to 12 modules in parallel can be controlled from one computer
- Up to 4 samples can be purified per module
- Up to 48 proteins can be purified within 14 hours using a two-step protocol
- Up to 24 proteins can be purified within 11 hours using a four-step protocol



## Acknowledgements

We are thankful to Dr. Darcy Birse at the Department of Biochemistry and Biophysics, Stockholm University, Stockholm, Sweden and TGN Biotech Inc., Québec, Canada, for providing us with GST-pur $\alpha$  protein. We are also grateful to Argonne National Laboratory, US, for providing us with APC234 and APC1040, two soluble protein targets of the Midwest Center for Structural Genomics ([www.mcsg.anl.gov](http://www.mcsg.anl.gov)).

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### Licensing information

Purification and preparation of fusion proteins and affinity peptides comprising at least two adjacent histidine residues may require a license under US patent 5,284,933 and US patent 5,310,663, including corresponding foreign patents (assignee: Hoffmann-La Roche Inc).

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