Automated on-column tag cleavage and multistep purification of $(histidine)_6$- and GST-tagged proteins

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Introduction

Affinity tags are commonly used to facilitate purification of recombinant proteins. For several downstream applications, the affinity tag needs to be removed from the target protein. ÄKTAxpress™ is a parallel chromatography system that allows automated multistep purification of, for example, $(histidine)_6$- and Glutathione S-Transferase (GST)-tagged proteins. All purification protocols that begin with ion exchange or affinity chromatography can be combined with on-column tag cleavage. The following results are presented:

- Optimization of cleavage conditions
- Automated on-column tag cleavage and further multistep purification of target proteins

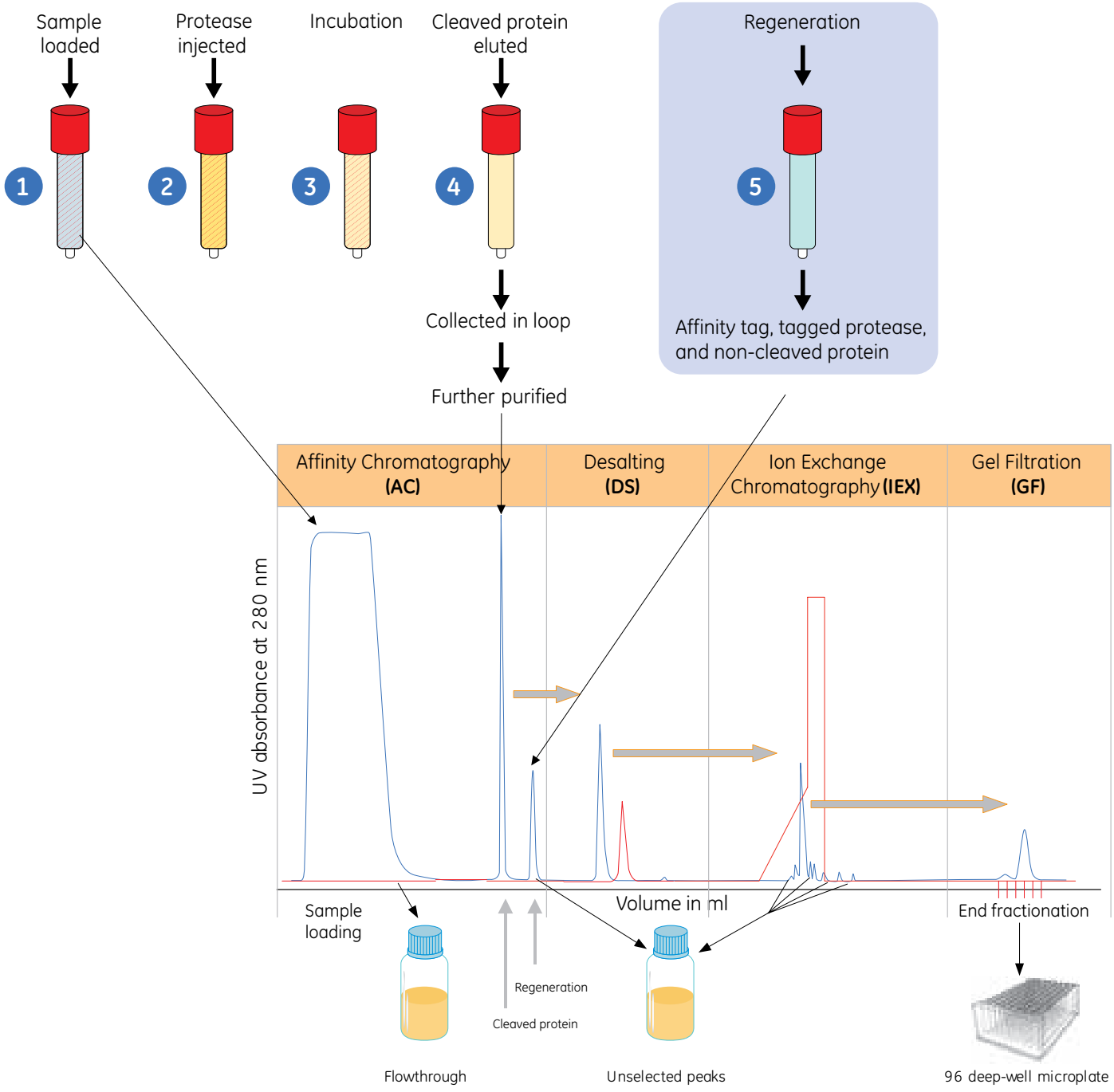
ÄKTAxpress provides:

- Automated multistep purification of affinity-tagged proteins or antibodies
- Method wizard software tool for easy creation of purification protocols
- Intelligent peak detection and collection in intermediate capillary loops
- Optional on-column tag cleavage
- Up to four samples can be purified per module
- Up to twelve system modules in parallel can be controlled from one computer

ÄKTAxpress has a modular design, with each module capable of purifying up to four proteins. ÄKTAxpress is available as a single module, or including several modules, such as the TWIN pack shown above.

Principles of multistep purification including on-column tag cleavage

First, cleavage is performed on an affinity or ion exchange column. Cleaved protein is further purified by different combinations of desalting, ion exchange and affinity chromatography, and gel filtration (size-exclusion chromatography).

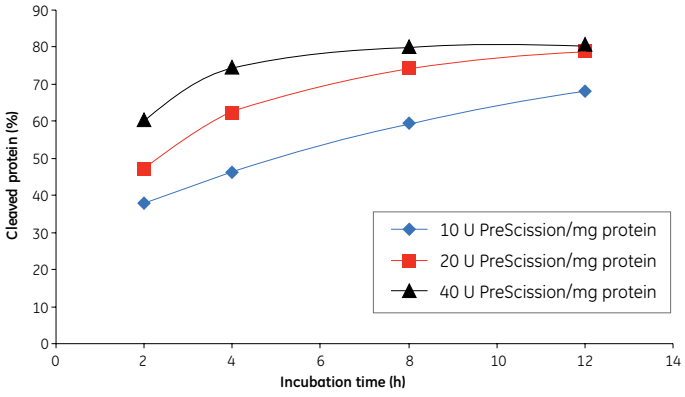


Note: This chromatogram is from a four-step purification including on-column cleavage using ÄKTAxpress software v1.0. In software v2.0, the regeneration of the affinity column is performed at the end of the purification scheme and its content collected in the fractionation plate.

Optimization of on-column tag cleavage

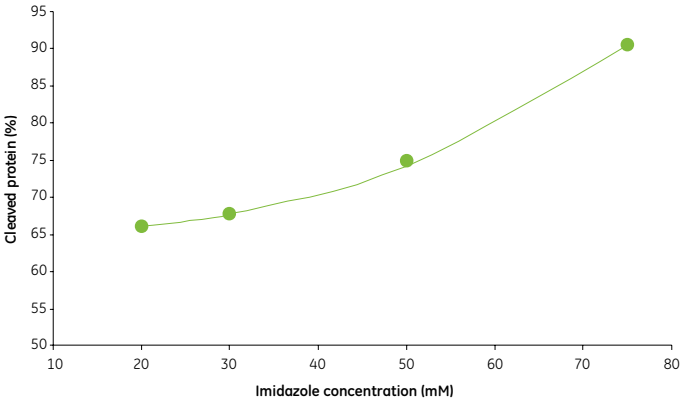
Effect of protease concentration and incubation time

On-column cleavage efficiency was measured by varying PreScission™ Protease concentration and incubation time using the GST-tagged protein GST-purα. Corresponding experiments were performed with AcTEV™ Protease and the $(histidine)_6$-tagged protein APC234 (data not shown).



Effect of imidazole concentration

For AcTEV Protease, the rate of on-column cleavage increased with higher imidazole concentration in the cleavage buffer. To avoid co-elution of AcTEV Protease and uncleaved protein with the cleaved target protein, the imidazole concentration was limited to 50 mM in further studies using ÄKTAxpress.



Materials and Methods

Effect of protease concentration and incubation time

Column:	GSTrap™ FF, 1 ml
Sample:	0.5 mg GST-purα/run
Protease:	10, 20, or 40 U of PreScission Protease (GST-tagged human rhinovirus 3C protease) per milligram GST-purα. Unit definition: One unit of PreScission Protease will cleave ≥ 90% of 100 µg of a test GST-tagged protein in cleavage buffer at 5°C for 16 h.
Binding and cleavage buffer:	50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.5
Elution buffer:	50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0
Temperature:	Cold room
System:	ÄKTAexplorer™ 100 with ÄKTA™ 3D Kit

Effect of imidazole concentration

Column:	HisTrap™ HP, 1 ml
Sample:	0.5 mg APC234/run
Protease:	200 U of AcTEV $[histidine]_6$-tagged tobacco etch virus (TEV) protease (Invitrogen) per milligram APC234. Unit definition: One unit AcTEV Protease cleaves ≥ 85% of 3 µg control substrate in 1 h at 30°C.
Binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5
Cleavage buffer:	50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5
Elution buffer:	50 mM Tris-HCl, 500 mM NaCl, 500 mM imidazole, pH 7.5
Temperature:	Room temperature
Incubation time:	4 h
System:	ÄKTAexplorer 100 with ÄKTA 3D Kit

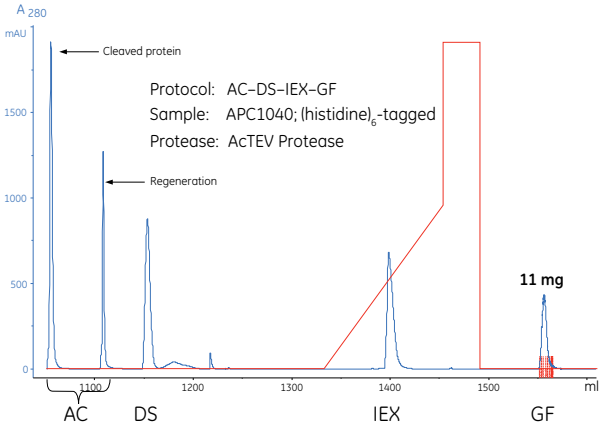
Results

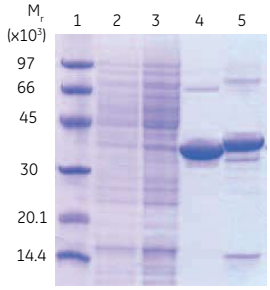
Based on the experiments described above, the following conditions were selected for on-column cleavage experiments using ÄKTAxpress:

Protease	Temperature	Incubation time	U protease/ mg protein	Imidazole concentration in cleavage buffer
PreScission Protease	cold room	8 h	20	not applicable
AcTEV Protease	room temperature	8 h	200	50 mM

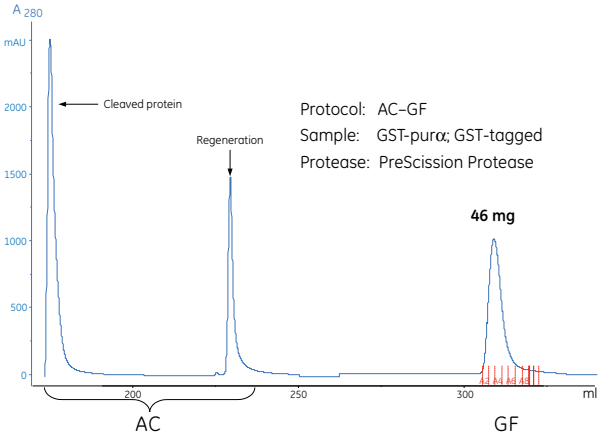
Automatic tag removal using ÄKTAxpress

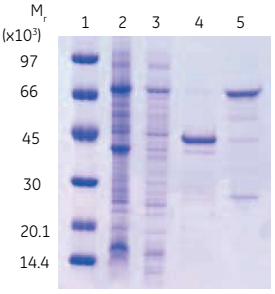
Two proteins – two proteases





Lane 1. Low Molecular Weight marker (LMW-SDS Marker Kit)
Lane 2. Start sample
Lane 3. Flowthrough
Lane 4. Purified, cleaved APC1040 (M_r 36.4 x 10^3) after AC–DS–IEX–GF
Lane 5. Reference: uncleaved APC1040 (M_r 38.9 x 10^3)





Lane 1. Low Molecular Weight marker (LMW-SDS Marker Kit)
Lane 2. Start sample
Lane 3. Flowthrough
Lane 4. Purified, cleaved GST-purα (M_r 35.2 x 10^3) after AC–GF
Lane 5. Reference: uncleaved GST-purα (M_r 61.6 x 10^3)

Two proteins were cleaved and purified using two different protocols. High amounts of pure cleaved protein were obtained using both PreScission Protease and AcTEV Protease.

Materials and Methods

Sample information

The $(histidine)_6$-tagged proteins APC234 and APC1040, and the GST-tagged protein GST-purα were used. All proteins were expressed in *E. coli*. After harvest, cell lysis was performed by sonication. The samples were clarified by centrifugation prior to sample loading.

Cleavage conditions

The optimized cleavage conditions were used.

Columns

AC: HisTrap HP, 5 ml (for $[histidine]_6$-tagged proteins)
AC: GSTrap HP, 5 ml (for GST-tagged proteins)
DS: HiPrep™ 26/10 Desalting
IEX: RESOURCE™ Q, 6 ml
GF: HiLoad™ 16/60 Superdex™ 75 pg

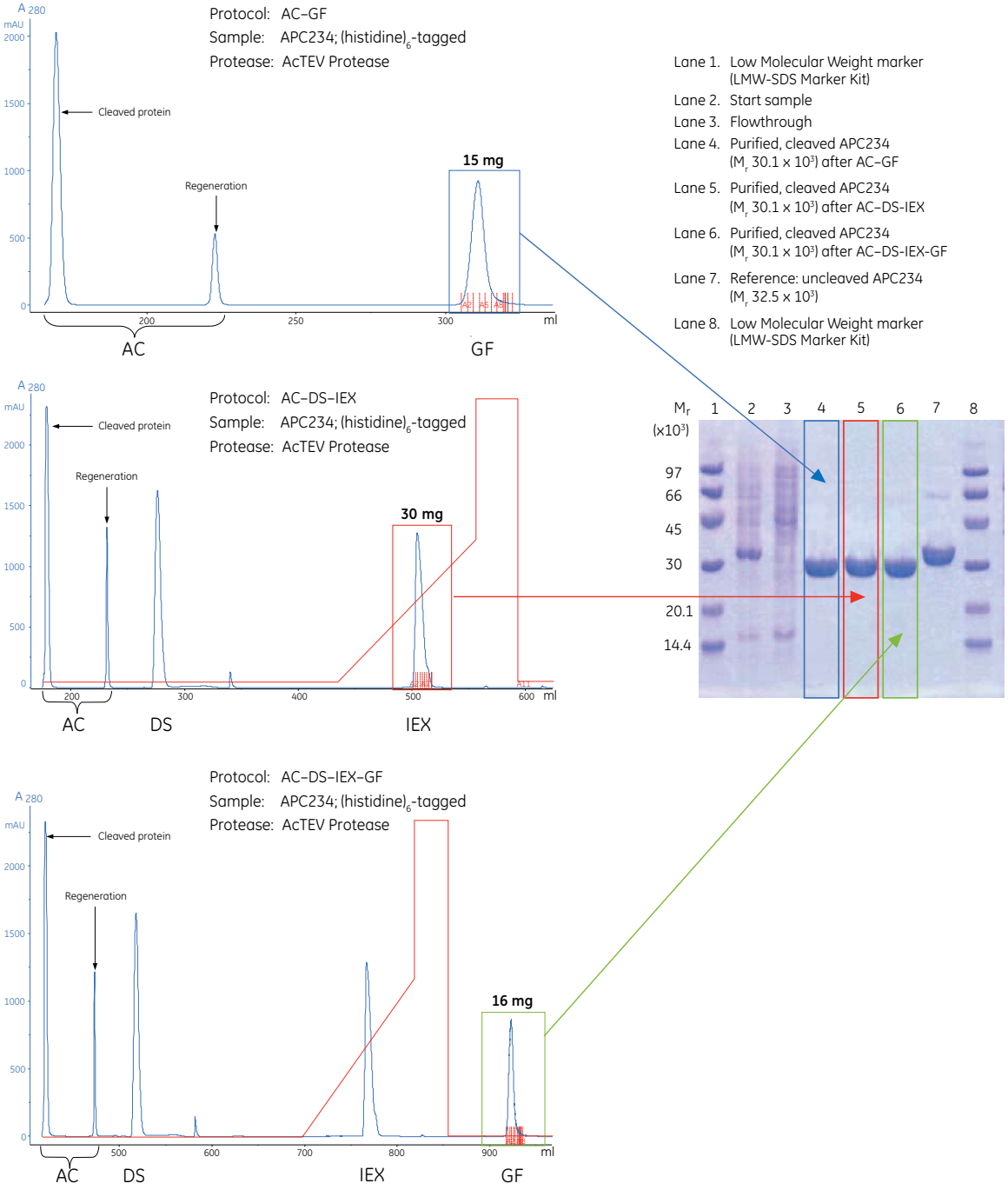
Buffers

AC (histidine) binding buffer:	50 mM Tris-HCl, 500 mM NaCl, 20 mM imidazole, pH 7.5
AC (histidine) cleavage buffer:	50 mM Tris-HCl, 500 mM NaCl, 50 mM imidazole, pH 7.5
AC (histidine) 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

The 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 polyacrylamide gels. Approximately 7.5 µg of protein was loaded per lane.

One protein – different multistep purification protocols



Lane 1. Low Molecular Weight marker (LMW-SDS Marker Kit)
Lane 2. Start sample
Lane 3. Flowthrough
Lane 4. Purified, cleaved APC234 (M_r 30.1 x 10^3) after AC–GF
Lane 5. Purified, cleaved APC234 (M_r 30.1 x 10^3) after AC–DS-IEX
Lane 6. Purified, cleaved APC234 (M_r 30.1 x 10^3) after AC–DS-IEX-GF
Lane 7. Reference: uncleaved APC234 (M_r 32.5 x 10^3)
Lane 8. Low Molecular Weight marker (LMW-SDS Marker Kit)

Three different multistep protocols were used to cleave and purify the same protein. Amounts of highly pure cleaved protein, sufficient for further applications such as structure determination, were obtained.

Conclusions

- Optimized conditions for on-column tag cleavage with PreScission Protease and AcTEV Protease were successful when using ÄKTAxpress multistep protocols.
- Automated on-column tag cleavage and purification of both $(histidine)_6$- and GST-tagged proteins were performed.
  - Yields of tens of milligrams of cleaved protein were obtained.
  - All processed proteins were of high purity.

Acknowledgements

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imagination at work

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