# Purification of GST-tagged protein using ÄKTA start

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Application note 29-0642-98 AC Chromatography systems

# Purification of GST-tagged protein using ÄKTA™ start

**ÄKTA start is an easy-to-use, reliable protein purification system. In this application note it is described how Erica, a post-doctoral researcher, used ÄKTA start to purify GST-tagged protein using a predefined method template, a GSTrap™ FF column and a Frac30 fraction collector.**

## Introduction

Affinity chromatography of tagged proteins is the most commonly used strategy for protein purification, and is an indispensable tool for Erica and researchers like her. She needs to purify glutathione S-transferase (GST)-tagged protein, as GST tags enable selective binding of target recombinant protein. GST tags also enhance solubility of the target protein and improve yield.

However, the manual purification method Erica used was resulting in inconsistency in terms of purity and yield and was consuming too much of her time. She needed a reliable single-step purification method.

Erica switched to ÄKTA start solution to automate the protein purification process in order to achieve reliable results and reproducibility across runs. She modified the predefined affinity chromatography method template available from the ÄKTA start instrument display. She used a prepacked GSTrap FF columns, designed for fast one-step purification of GST-tagged proteins. She loaded the pretreated bacterial lysates directly onto the GSTrap column and eluted under mild denaturing conditions.

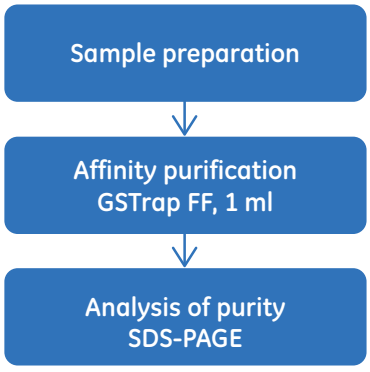


**Fig 1.** Workflow used for purification of target GST-tagged protein

## Methods

Erica developed the purification protocol from the affinity chromatography method template present in ÄKTA start and followed the workflow illustrated in Figure 1. She used the interactive touchscreen on the instrument to select the affinity template (Fig 2) and modified the run parameters to suit her needs (Table 1). She plugged in a USB memory stick to save the results. Erica prepared the sample by resuspending *E. coli* cells containing the GST-tagged protein in 10 mM sodium phosphate, 150 mM NaCl, pH 7.5. She equilibrated the column with 5 column volumes (CV) of binding buffer (10 mM sodium phosphate buffer, 150 mM NaCl, pH 7.5) and loaded 5 ml of sample onto a GSTrap FF 1 ml column. The ÄKTA start pump was used for sample loading at a flow rate of 0.5 ml/min to allow enough time for protein to bind to the media.

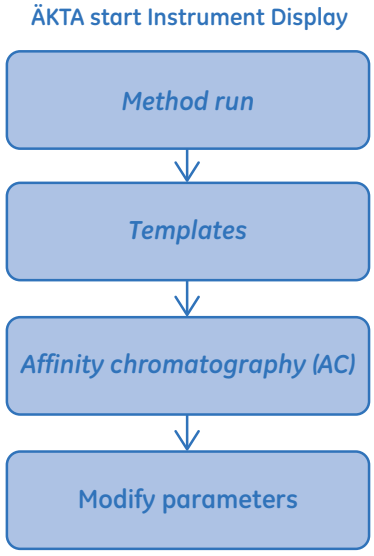


**Fig 2.** Screen selection for modifying the affinity template in ÄKTA start (screen choices are italicized).

After sample loading, she washed the column with 10 CV of binding buffer at 0.5 ml/min flow rate to remove unbound proteins and other contaminants. Erica eluted the bound protein using 5 CV of 100% elution buffer (10 mM sodium phosphate buffer, 150 mM NaCl, 10 mM reduced glutathione, pH 7.5) in a single step, collecting 1 ml fixed volume fractions using a Frac30 fraction collector. She then re-equilibrated the column with 5 CV of binding buffer.

*Create and store chromatography methods in ÄKTA start.*

Erica monitored the run using the real-time UV trace (280nm), conductivity and pressure values from the instrument display. She viewed the bitmap (.bmp) image file generated from ÄKTA start to identify the fractions of interest. After pooling the protein fractions she assessed the purity of protein by 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

**Table 1.** Standalone method flow. Run parameters are shown as displayed on the ÄKTA start display screen

| | |
|---|---|
| **Screen 1 run parameters** | |
| Column volume | 1.0 ml |
| Flow rate | 0.5 ml/min |
| Pressure limit | 0.3 MPa[1] |
| Save result to USB | ACO1 |
| **Screen 2 run parameters** | |
| Sample from | Pump |
| Sample volume | 5.0 ml |
| Equilibration volume | 5.0 CV[2] |
| Wash unbound volume | 10.0 CV[2] |
| **Screen 3 run parameters** | |
| Elution option | Isocratic |
| Conc B | 100% B |
| Elution volume | 5.0 CV[2] |
| Fractionation volume | 1.0 ml |

[1] 0.3 MPa = 3 bar (43.5 psi)
[2] Column volume

*Results from ÄKTA start can be easily imported to UNICORN™ start using a USB memory stick.*

## Result

In this study Erica purified GST-tagged protein in a single purification step using the affinity chromatography method template and a GSTrap FF 1 ml column. The result that was saved as a .bmp image file (Fig 3A) on the USB memory stick provided flexibility for Erica to view and print the report using any image viewing software.

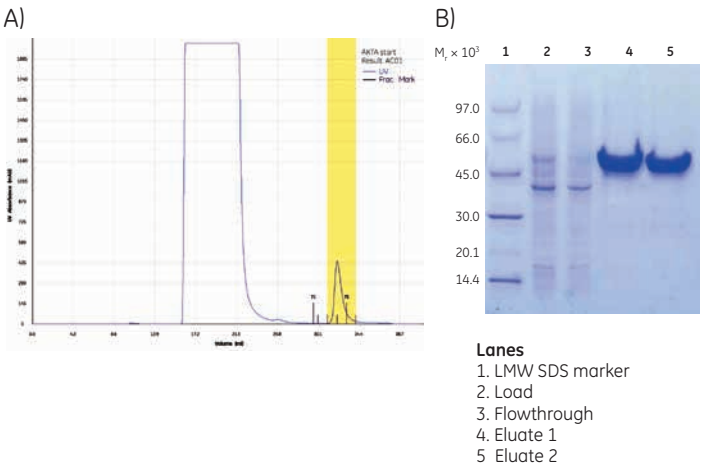


**Fig 3.** A) Chromatogram showing the purification profile of the GST-tagged protein on ÄKTA start. The highlighted (yellow) area represents the pooled fractions. The image file displays the UV curve with fraction marks. B) 12.5% SDS-PAGE profile of the purified protein factions.

Purification of the protein using ÄKTA start together with the prepacked column gave her high purity (> 95%) as shown by SDS-PAGE (Fig 3B). To further analyze the data she imported the result file from the USB memory stick into UNICORN start 1.0 control software running on a computer in a neighboring lab (Fig 4). She used the Evaluation module of the software to perform peak integration and to create a PDF report. Automating the purification process reduced Erica's need for manual intervention, improved reproducibility, and reduced her time and effort compared with her previous manual purification protocols.

*When using UNICORN start, UV level- or slope-based fractionation can be performed.*

| | |
|---|---|
| *Column*: | GSTrap FF 1 ml |
| *Sample*: | Cell extract resuspended in 10 mM sodium phosphate buffer, 150 mM NaCl, pH 7.5 |
| *Sample volume*: | 5 ml |
| *Binding buffer*: | 10 mM sodium phosphate buffer, 150 mM NaCl, pH 7.5 |
| *Elution buffer*: | 10 mM sodium phosphate buffer, 150 mM NaCl, 10 mM reduced glutathione, pH 7.5 |
| *Flow rate*: | 0.5 ml/min |
| *Gradient*: | 100% step |
| *System*: | ÄKTA start and Frac30 fraction collector |
| *Detection*: | UV (280 nm) |

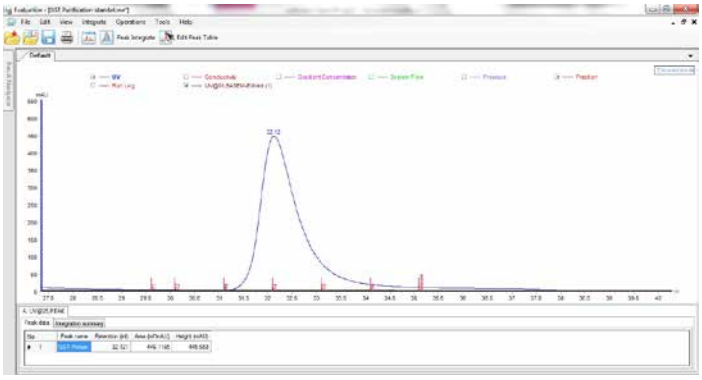

**Fig 4.** Chromatogram showing the peak of interest with retention time, after performing peak integration using UNICORN start Evaluation module. Detailed peak data is provided below the graph.

## Summary

Erica modified the affinity chromatography template in ÄKTA start to reliably obtain high quality GST-tagged protein, suitable for use in her research work. Frac30 fraction collector facilitated the collection of fixed volume fractions without any manual intervention. The affinity template can be used for the purification of any tagged protein and, as Erica demonstrated, can be customized depending on purification requirements.

## Ordering information

| Product | Quantity | Code number |
|---|---|---|
| ÄKTA start | 1 | 29-0220-94 |
| Frac30 Fraction collector | 1 | 29-0230-51 |
| UNICORN start software | 1 | 29-0187-51 |
| GSTrap FF | 5 × 1 ml | 17-5130-01 |
| LMW-SDS Marker Kit | 1 | 17-0446-01 |

### *Related literature*

| Product | Code number |
|---|---|
| Purification of N-terminal histidine-tagged protein using ÄKTA start, Application note | 29-0642-77 |
| Depletion of albumin from serum samples using ÄKTA start, Application note | 29-0642-95 |
| Purification of antibodies using ÄKTA start and HiTrap Protein G HP column, Application note | 29-0643-02 |

For local office contact information, visit
**www.gelifesciences.com/contact**

www.gelifesciences.com/AKTAstart

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work



GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5
D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Japan Corporation
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan

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