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Application Note

► Automated purification of His-tagged β-galactosidase

Category Bioanalytics
Matrix Enzymes
Method FPLC
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Analytes β-galactosidase
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Summary

This application note describes an automated method using BioFox 40 IDAlow loaded with Ni^{2+} and a desalting column for a two-step automated isolation of a recombinant β-galactosidase. The high metal-ion capacity of the media and the automation of the method make it well-suited for a wide range of research laboratories and for the industry.

Introduction

The hydrolase enzyme β-galactosidase catalyzes the hydrolysis of β-galactosides into monosaccharides. It is an essential enzyme in the human body and commonly used in molecular biology as a reporter marker in various applications.

Several IMAC applications have been developed for the purification of the recombinant β-galactosidase[1]. Ni-NTA agarose is the most common used resin for IMAC preparations. In comparison to BioFox 40 IDAlow loaded with Ni^{2+}, Ni-NTA has a lower pressure resistance followed by a slower filling process and a poorer resolution.

BioFox 40 IDAlow agarose is produced from highly purified agarose using a proprietary cross-linking method that results in a highly porous and physically stable agarose matrix. The ligand IDA is comparable to NTA, but the capacity of binding metal-ions is much lower for IDAlow because of the final spherical interaction between the proteins of interest.

In this study, the resin BioFox 40 IDAlow loaded with Ni^{2+} was pressure-filled in a Bioline HR glass column and connected to a Bioline protein purification system as well as a desalting column. In an automated run, the isolation of His-tagged β-galactosidase will be shown.

Experimental: Sample preparation

Kluyveromyces lactis cells were cultured and the genomic DNA isolated from the harvested cells. The *LAC4*-coding DNA fragment was amplified from *K. lactis* genomic DNA by PCR. *K. lactis β-galactosidase* gene was expressed in *E.coli* as a His-tagged recombinant enzyme.

Method parameters

Column	BioFox 40 IDAlow (Ni^{2+}), 11 ml, 10 x 300 mm			
Eluent A	50 mM Na/K-phosphate buffer (pH 6.5) + 500 mM NaCl			
Eluent B	50 mM Na/K-phosphate buffer (pH 6.5) + 500 mM NaCl + 500 mM imidazole			
Eluent C	20 mM Tris-buffer (pH 7.2)			
Gradient	**t [min]**	**% A**	**% B**	**% C**
	0.0	96	4	0
	20.0	96	4	0
	20.1	96	4	0
	35.0	96	4	0
	35.1	0	100	0
	42.5	0	100	0
	42.6	0	0	100
	120.0	0	0	100
Flow rate	0.8 ml/min			
m [injection]	200 mg total protein			
T [column]	RT			
Detection	UV at 280 nm			
Run time	100 min			

Results

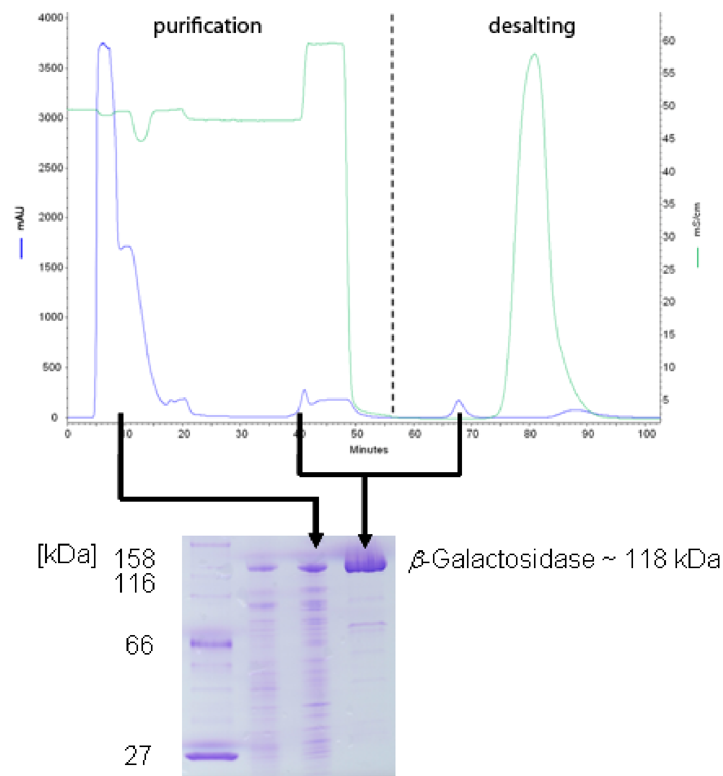

Fig. 1

Purification of recombinant, His_6-tagged β-galactosidase from *Kluyveromyces lactis.*

Using a KNAUER Bioline biochromatography system and an HR glass column filled with BioFox 40 IDAlow loaded with Ni^{2+}, recombinant His-tagged β-galactosidase was successfully separated from *E.coli* in an automated run (Fig. 1). After the desalting step, the analysis of the SDS-PAGE with Comassie Blue staining allowed for the identification of β-galactosidase using a molecular weight marker (lane 1). The yields of the separated product before desalting (lane 3) and after desalting (lane 4) were compared to the extract and showed a increase of β-galactosidase after desalting (lane 4).

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Conclusion

The recombinant β-galactosidase expressed in *E.coli* is a His-tagged fusion protein and was purified by Ni- BioFox 40 IDAlow agarose affinity chromatography. The separation on an IMAC medium involving a final desalting step, was run on a Bioline protein purification system with an HR glass column. This fully automated method produced in a highly-purified protein fraction.

References

1 Chang Sup Kim et al. Biotechnology Letters, Vol. 25, 1769-1774 (2003)

Authors

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Physical properties of recommended column

Stationary phase	BioFox 40 IDAlow [loaded with Ni^{2+}]
Specific surface area	320 m^2/g
Particle size	40 µm (32-60 µm]
Metal-ion capacity	10-20 µequ Ni^{2+}/ml]
Order number resin	Y4025
HR Bioline glass column	10 x 300 mm
Order number	A71110

Recommended instrumentation

The biochromatography run was performed on a KNAUER Bioline system, equipped with a gradient pump S1050, degasser unit S5050, UV detector, conductivity detector, and a fraction collector.

Description	**Order No.**
Smartline Pump 1050	A50351-1
Smartline Manager 5050	A5331-1
SmartMix 350	A5351
Bioline Assistant 6000	A5004V316
Smartline UV Detector 2520	A5150
3 mm flow cell	A4132
Smartline Conductivity Monitor 2900	A70090
Smartline Fraction Collector	A53712
Bioline affinity module	A7004V322
Bioline MPLC Rack	A70190
Bioline Thermostate	A70050
ChromGate software	A1456-8/A1470-8/A1512-8

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