



Nov 04, 2025

Expression and Purification of AtGa3ox1, a Key Gibberellin 3-Oxidase from *Arabidopsis thaliana*

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwbxd9vmk/v1>

Isabel A. Abreu¹, Joana Marques¹

¹ITQB NOVA



Isabel A. Abreu

ITQB NOVA

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwbxd9vmk/v1>

Protocol Citation: Isabel A. Abreu, Joana Marques 2025. Expression and Purification of AtGa3ox1, a Key Gibberellin 3-Oxidase from *Arabidopsis thaliana*. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.e6nvwbxd9vmk/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 07, 2025

Last Modified: November 04, 2025

Protocol Integer ID: 123986

Keywords: Gibberellins, AtGa3ox1, gibberellin biosynthesis, GA₄, GA₁, protein expression, Escherichia coli, recombinant protein, TEV protease cleavage, His-tag purification, size-exclusion chromatography, FLAG-tag, plant hormone metabolism, oxidase from arabidopsis thaliana gibberellin, purification of atga3ox1, heterologous expression of arabidopsis thaliana ga, arabidopsis thaliana gibberellin, arabidopsis thaliana ga, direct biosynthesis, step in the biosynthesis, biosynthesis, direct biosynthesis of the bioactive, subsequent purification of the recombinant protein, induction of flowering, atga3ox1, oxidase, key stages of plant development, nature of the purified protein, detailed steps for gene cloning, flowering, purity protein suitable for biochemical study, protein expression, purified protein, seed germination, plant development, recombinant protein, enzyme, gene cloning, affinity purification, at1g15550, purity protein, phytohormone

Funders Acknowledgements:

"GREEN-IT "Biorecursos para a Sustentabilidade""

Grant ID: UIDP/04551/2020, <https://doi.org/10.54499/UIDP/04551/2020>

"GREEN-IT "Biorecursos para a Sustentabilidade""

Grant ID: UIDB/04551/2020, <https://doi.org/10.54499/UIDB/04551/2020>

FCT

Grant ID: 2020.06917.BD

Abstract

Gibberellins (GAs) are phytohormones involved in key stages of plant development such as seed germination, maturation, and the induction of flowering (reviewed in [1]). GA 3-oxidases catalyse the final and rate-limiting step in the biosynthesis of bioactive GAs [1]. *AtGa3ox1* is an enzyme involved in the direct biosynthesis of the bioactive gibberellins GA₄ and GA₁. This protocol describes the heterologous expression of *Arabidopsis thaliana* GA 3-oxidase 1 (*AtGa3ox1*, At1g15550) in *Escherichia coli* and the subsequent purification of the recombinant protein using affinity and size-exclusion chromatography. The protocol includes detailed steps for gene cloning, protein expression in *E. coli* Rosetta (DE3) pLysS, affinity purification via His-tag chromatography, TEV protease cleavage for tag removal, and size-exclusion chromatography to obtain high-purity protein suitable for biochemical studies. The nature of the purified protein was confirmed by mass-spectrometry analysis.

Guidelines

Columns should be cleaned according to the manufacturer's instructions and stored in 20% ethanol when not in use.

All buffers must be filtered (0.22 µm) or degassed prior to use.

All chromatography steps were carried out at 4 °C.

Materials

	Primer Name	Primer sequence from 5' to 3'
	GA3ox1_a582g_Rv	tggggtcagcgaagaggacattgaatggg cc
	GA3ox1_a582g_Fwd	ggcccattcaatgtcctcttcgctgacccca
	BpilFs5_Ga3ox1_F	CACCACAGAAGACGAGCTTTGCC TGCTATGTTAACAGATGTG
	BpilFs7_Ga3ox1_RV	CACCACAGAAGACGAAGCGTCAT TCTTCTCTGTGATTCT

Table 1: Primer list

Plasmids:

pet28g

E. coli Strain:

Rosetta(DE3) pLysS (invitrogen)

Lysogeny broth (LB) Medium: per 1 L: 10 g tryptone, 5 g yeast extract, 10 g NaCl

Antibiotics:

Kanamycin (Kan): stock concentration 50 mg/mL

Chloramphenicol (Cam): stock concentration 30 mg/mL

Enzymes:

⊗ DpnI (10 U/μL) Thermo Fisher Catalog #ER1701

⊗ Pfu Ultra HF DNA Pol Agilent Technologies

⊗ Phusion GC Buffer Pack Thermo Fisher Catalog #F519L

⊗ Bsal-HFv2 New England Biolabs Catalog #R3733S

⊗ Bpil (FastDigest) Thermo Fisher Scientific Catalog #FD1014

Buffers:

Buffer A:

20 mM KPi pH 7.5

250 mM NaCl

20 mM imidazole

5% glycerol

1 Tablet of cOmplete, EDTA-free (Protease Inhibitor Cocktail) (PIC)
 DNase (the tip of a small weighing spoon, approximately)
 5 mM MgCl₂

Buffer B:

20 mM KPi pH 7.5
 250 mM NaCl
 500 mM imidazole
 5% glycerol

Running buffer (preparative SEC column):

20 mM KPi pH 7.5
 250 mM NaCl
 5% glicerol

Buffer D:

20 mM Tris-HCl pH 7.5
 100 mM NaCl
 1 mM EDTA
 1 mM DTT

Buffer E:

20 mM KPi pH 7.5
 100 mM NaCl

Purification equipment:

Equipment	
ÄKTA pure	NAME
Protein purification system	TYPE
Cytiva	BRAND
29046665	SKU
https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665 ^{LINK}	

Columns:

Equipment	
HisTrap HP His tag protein purification columns	NAME
protein purification columns	TYPE
Cytiva	BRAND
17524801	SKU
https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/affinity-tagged-protein/hisrap-hp-histidine-tagged-protein-purification-columns-p-00250	LINK
5mL	SPECIFICATIONS

Equipment	
HiLoad Superdex 200 pg preparative SEC column	NAME
Protein Purification column	TYPE
Cytiva	BRAND
28989336	SKU
https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/size-exclusion/hiload-superdex-200-pg-preparative-size-exclusion-chromatography-columns-p-06283	LINK
16/60	SPECIFICATIONS

Equipment

PD-10 desalting columns packed with Sephadex G-25 resin

NAME

Desalting column

TYPE

Cytiva

BRAND

17085101

SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/desalting-and-buffer-exchange/sephadex-g-25-in-pd-10-desalting-columns-p-05778?srltid=AfmBOooJv9gajWJJ0zO9u63gpc4O6iqrFBAJp3ig50ykPYVk4dqle0Ob>

LINK

Concentrator:

Equipment

Ultra Centrifugal Filter, 10 kDa MWCO

NAME

Centrifugal concentrator

TYPE

Amicon®

BRAND

UFC9010

SKU

https://www.sigmaaldrich.com/PT/en/product/mm/ufc9010?utm_source=google&utm_medium=cpc&utm_campaign=21862449245&utm_content=166991182302&gad_source=1&gad_campaignid=21862449245&gbraid=0AAAAAD8kLQR7nQr3a8zGpW1yo7XERDsJ-&gclid=Cj0KCQjwgIXCBhDBARIsAELC9Zh8Ao

LINK



Safety warnings



It's important to set the pressure alarm for each column before use.

Ethics statement

Not applicable.

Before start

Users should be familiar with bacterial culture techniques and standard protein purification methods. All buffers should be prepared fresh, filtered (0.22 μm), and degassed to ensure optimal chromatography performance. Chromatography steps are performed at 4 °C to preserve protein stability. The total time for protein production and purification is approximately 3–4 days, depending on the scale and equipment availability.

Cloning of AtGa3ox1

- 1 The AtGa3ox1 gene was cloned into the protein expression vector pET28g [2]. Prior to cloning, the gene was domesticated for Golden Gate assembly by introducing a point mutation (A582G) [3], using the primers *Ga3ox1_a582g_fwd* and *Ga3ox1_a582g_rv* (see primer list). The domesticated gene was then amplified using *BpilFs5_Ga3ox1_1F* and *BpilFs7_Ga3ox1_1076R*, and cloned into the Level 0 Golden Gate vector piCH41276. The following modules were assembled into the final expression construct pET28g-AtGa3ox1: *p028g13:8His-GB1*, *p028g34:TEVs*, *p028g45:3xFLAG*, *piCH41276-AtGa3ox1*, and *pET28g* [2,3].



Figure 1. Schematic representation of Ga3ox1 tagged with an N-terminal 6×His tag and a C-terminal 3×FLAG epitope.

Bacterial cell growth

- 2 *E. coli* Rosetta (DE3) pLysS cells transformed with pET28g-AtGa3ox1 were grown at 37 °C, shaking at 180 rpm, in LB medium (per 1 L: 10 g tryptone, 5 g yeast extract, 10 g NaCl), supplemented with 50 µg/mL kanamycin and 30 µg/mL chloramphenicol, until the culture reached an OD₆₀₀ of 0.8–1.0. Cultures were grown in 5 L baffled-bottom Erlenmeyer flasks, each containing 2.5 L of culture volume. 
- 3 Protein expression was induced with 400 µM IPTG, and the culture was incubated for 5 hours at 30 °C, shaking at 180 rpm, before harvesting. 
- 4 Cells were collected by centrifugation at 16,000 × g for 1 hour at 4 °C. 

Cell lysis

- 5 The pellet obtained from 2.5 L of culture (approximately 6.75 g wet weight) was resuspended in 45 mL of Buffer A (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 20 mM imidazole; and 5% glycerol) with 1 tablet of cComplete, EDTA-free Protease Inhibitor Cocktail (PIC), 5 mM MgCl₂, and DNase (the tip of a small weighing spoon, approximately).
- 6 Cells were lysed using a French press with three passes at 15,000 psi.

7 The lysate was centrifuged at $20,000 \times g$ for 1 hour and 30 minutes at 4 °C. The supernatant was collected, and the pellet was discarded.

8 The collected supernatant was filtered through a 0.22 µm cellulose nitrate syringe filter to obtain the protein extract for subsequent purification steps.

Protein Purification - Step 1: HisTrap

9 All chromatography steps were carried out at 4 °C. A HisTrap HP column (Cytiva) was equilibrated, and the protein extract was loaded using a peristaltic pump (see Materials) at a flow rate of 5 mL/min. All subsequent chromatographic steps were performed on an ÄKTA pure system (see Materials) at a flow rate of 1 mL/min, with a pressure limit of 0.5 MPa.

Equipment

HisTrap HP His tag protein purification columns

NAME

protein purification columns

TYPE

Cytiva

BRAND

17524801

SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/affinity-tagged-protein/hisrap-hp-histidine-tagged-protein-purification-columns-p-00250>

LINK

5mL

SPECIFICATIONS

Equipment

ÄKTA pure

NAME

Protein purification system

TYPE

Cytiva

BRAND

29046665

SKU

<https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665>

LINK

9.1 **Column preparation:** The column was first washed with 5 column volumes (25 mL) of water, followed by equilibration with 5 column volumes of binding buffer.

9.2 **Column loading:** The protein extract (45 mL) was loaded onto the column by recirculating it 2–3 times through the column to maximize protein binding.

10 **Column washing:** The loaded column was then connected to the ÄKTA pure system, and non-bound proteins were removed by washing with Buffer A until the baseline stabilized.

Note

Note: This step ensures the removal of contaminating endogenous E. coli proteins.

11 **Elution:** Proteins were eluted using an imidazole gradient (0% to 100% Buffer B) by mixing Buffer A and Buffer B (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 500 mM imidazole; and 5% glycerol) over 15 column volumes (75 mL). Eluted fractions were collected in 2 mL volumes.

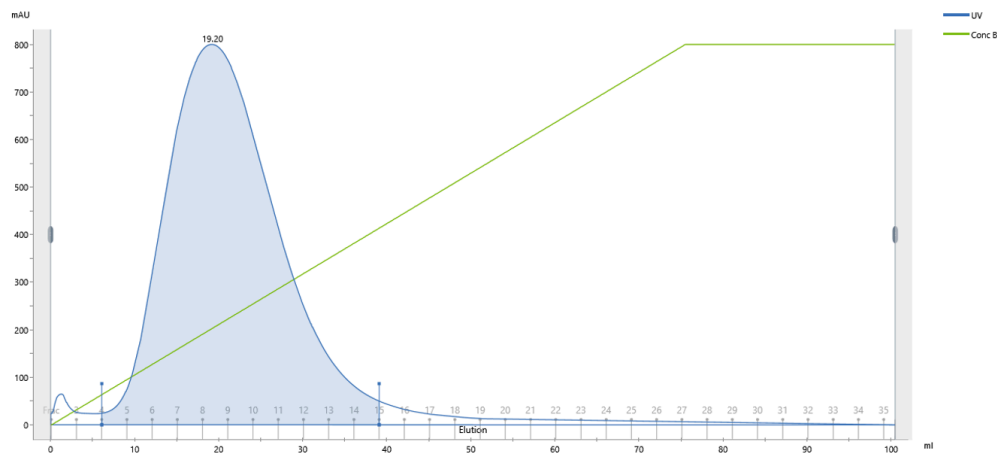


Figure 2. Purification of 6×His-tagged *AtGa3ox1* using a HisTrap HP column (Cytiva). Protein extract from *E. coli* Rosetta (DE3) pLysS expressing 6×His-*AtGa3ox1* was loaded onto a HisTrap HP column and purified on an ÄKTA pure system at a flow rate of 1 mL/min. The column was washed with Buffer A (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 20 mM imidazole; 5% glycerol) and proteins were eluted using a linear gradient of Buffer B (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 500 mM imidazole; and 5% glycerol).

12 The injected, flow-through, and collected fractions were run in a 10% SDS gel.

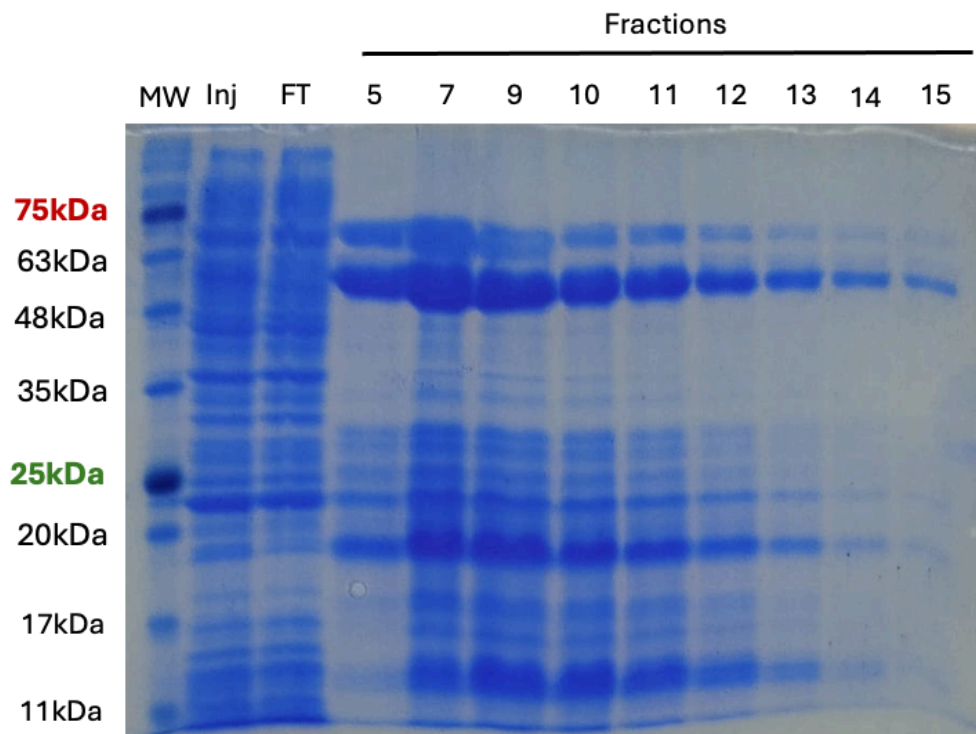


Figure 3. SDS–PAGE analysis of eluted fractions containing 6×His-tagged *AtGa3ox1*. The soluble fraction from *E. coli* Rosetta (DE3) pLysS expressing *AtGa3ox1* was loaded onto the ÄKTA pure system (injected, Inj). Non-bound proteins were collected in the flow-through (FT) after washing with Buffer A (20mM potassium phosphate, pH7.5; 250mM NaCl; 20mM imidazole; 5% glycerol). Elution was performed with a linear gradient of Buffer B (20mM potassium phosphate, pH7.5; 250mM NaCl; 500mM imidazole; 5% glycerol). Eluted fractions 5 to 15 were analysed by SDS–PAGE and stained with Coomassie Blue (Blue Safe, NZYTech). Molecular weight marker (MW) and corresponding sizes are indicated on the left.

- 13 Fractions 4 to 14 were pooled and concentrated to a final volume of 2 mL for injection into the HiLoad Superdex 200 pg preparative SEC column. Concentration was performed using an Amicon Ultra-15 centrifugal filter unit (10 kDa cutoff) by centrifugation at $4,000 \times g$ at 4°C in a swing-bucket benchtop centrifuge. Centrifugation was done in 10-minute increments until the desired volume was reached.

Note

Note: No precipitate was observed during the concentration process.

Equipment

Ultra Centrifugal Filter, 10 kDa MWCO

Centrifugal concentrator

Amicon®

UFC9010

https://www.sigmaaldrich.com/PT/en/product/mm/ufc9010?utm_source=google&utm_medium=cpc&utm_campaign=21862449245&utm_content=16&gclid=Cj0KCQjwgIXCBhDBARIsAELC9Zh8Ao

Protein purification - Step 2: Hiload Superdex 200

- 14 All chromatography steps were performed on an ÄKTA pure system (see Materials) at 1mL/min, with a pressure limit of 0.5 MPa, and maintained at 4 °C. The HiLoad Superdex 200 pg preparative SEC column (Cytiva) was equilibrated, and the concentrated protein sample was injected at a flow rate of 1 mL/min.

Equipment

HiLoad Superdex 200 pg preparative SEC column

NAME

Protein Purification column

TYPE

Cytiva

BRAND

28989336

SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/size-exclusion/hiload-superdex-200-pg-preparative-size-exclusion-chromatography-columns-p-06283>

LINK

16/60

SPECIFICATIONS

- 14.1 **Column preparation:** The column was first washed with 5 column volumes (620 mL) of water, followed by equilibration with 5 column volumes of running buffer (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 5% glycerol; and 0.02% Tween 20).

Note

Note: To prevent non-specific ionic interactions between proteins and the column matrix, the running buffer should contain at least 0.15M salt.

- 14.2 **Column loading:** The concentrated pooled fractions (fractions 4 to 14) from the HisTrap purification (total volume: 2 mL) were injected into the column via the ÄKTA pure system.

Note

Note: Sample volume is critical for optimal separation resolution.

- 15 **Elution:** Proteins were eluted with running buffer (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 5% glycerol; 0.02% Tween 20), and fractions of 1 mL were collected.

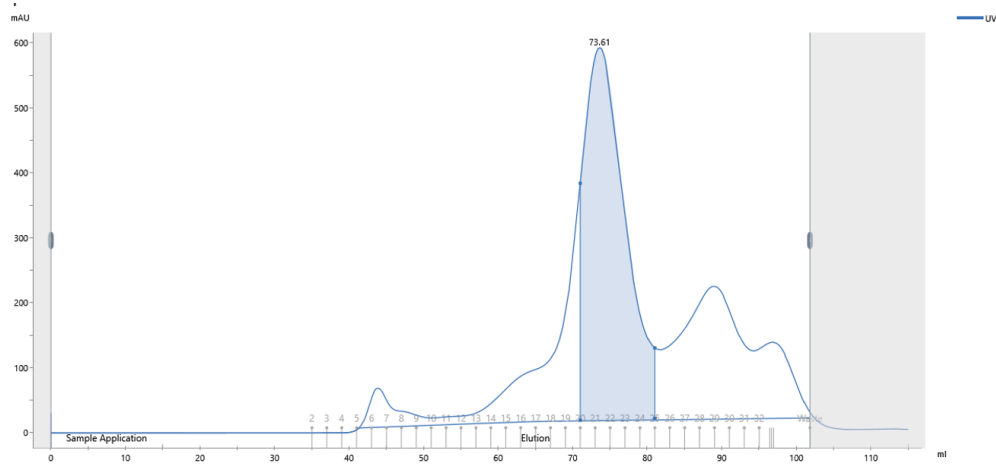


Figure 4. Purification of 6×His-tagged *AtGa3ox1* using preparative size-exclusion chromatography (SEC).

Pooled fractions from the HisTrap purification were concentrated and injected into a HiLoad Superdex 200 pg preparative SEC column (Cytiva). The running buffer consisted of 20 mM potassium phosphate (pH 7.5), 250 mM NaCl, 5% glycerol, and 0.02% Tween 20. Eluted fractions were collected in 1 mL volumes.

- 16 The injected sample and collected SEC fractions were analyzed by SDS-PAGE on a 10% polyacrylamide gel.

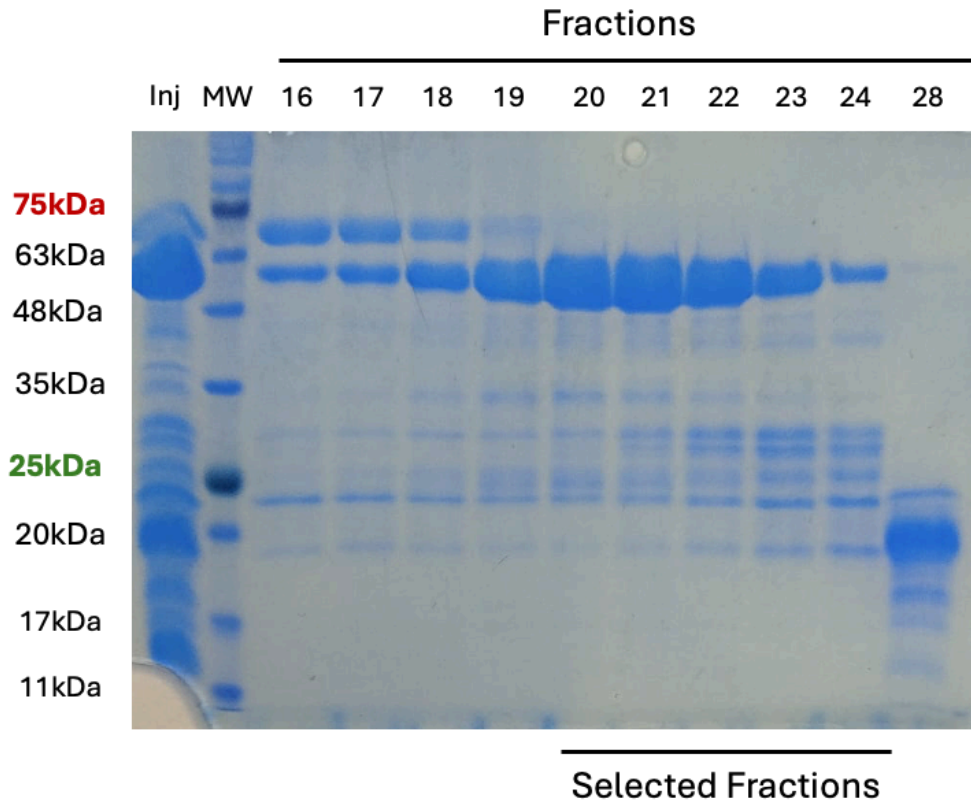


Figure 5. SDS–PAGE analysis of 6×His-tagged *AtGa3ox1* eluted from a preparative SEC column.

Pooled fractions from the HisTrap purification were concentrated and injected into the HiLoad Superdex 200 pg column (inj). The running buffer consisted of 20mM potassium phosphate (pH7.5), 250mM NaCl, 5% glycerol, and 0.02% Tween 20. Eluted fractions 16 to 28 were analysed by SDS–PAGE on a 10% gel and stained with Coomassie Blue (Blue Safe, NZYTech). Molecular weight marker (MW) and corresponding sizes are shown to the left of the gel. Fractions 20 to 24 were selected for further purification.

The selected fractions (20 to 24) were concentrated to 2.5 mL, as previously described, for loading onto a PD-10 desalting column.

Note: No precipitate was observed during concentration.

17

To prepare the protein sample for TEV protease cleavage, the buffer was exchanged.

Column preparation: The PD-10 desalting column was equilibrated with a total of 25 mL of cleavage buffer (20 mM Tris-HCl, pH 7.5; 100 mM NaCl; 1mM EDTA; 1mM DTT).

Equipment

PD-10 desalting columns packed with Sephadex G-25 resin

NAME

Desalting column

TYPE

Cytiva

BRAND

17085101

SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/desalting-and-buffer-exchange/sephadex-g-25-in-pd-10-desalting-columns-p-05778?srsId=AfmBOooJv9gajWJJ0zO9u63gpc4O6iqrFBAJp3ig50ykPYVk4dqle0Ob>

LINK

17.1 **Column loading:** A total of 2.5 mL of the selected and concentrated fractions (from Step 16) was slowly and directly loaded onto the PD-10 column.

17.2 **Elution:** Proteins were eluted by centrifugation at $1,000 \times g$ for 2 minutes at 4°C to a final volume of 1.5 mL.



Protein Purification - Step 3: Affinity tag removal by TEV protease

18 To remove the 6×His tag, the purified protein was treated with TEV protease. The protease was added at a 1:20 ratio (w/w) relative to the target protein. In this case, 0.46 mg of TEV protease was added to 9.16 mg of 6×His-(TEV cleavage site)-3×FLAG-Ga3ox1. The digestion was carried out overnight at 4°C .



19 Purification of the cleaved protein was performed using a reverse HisTrap approach. After TEV cleavage, the reaction mixture contained the target protein, 7×His-tagged TEV protease, and the free 6×His tag. To isolate the cleaved protein, the mixture was applied to a 5 mL HisTrap column (Cytiva), allowing the His-tagged components to bind to the resin while the cleaved, untagged protein eluted in the flow-through.

Protein Purification - Step 4: Reverse HisTrap

20 All chromatography steps were performed at 4°C . Subsequent steps were carried out on an ÄKTA pure system (see Materials) at a flow rate of 5 mL/min, with a pressure limit of 0.5 MPa.

Equipment

HisTrap HP His tag protein purification columns

NAME

protein purification columns

TYPE

Cytiva

BRAND

17524801

SKU

<https://www.cytivalifesciences.com/en/us/shop/chromatography/prepacked-columns/affinity-tagged-protein/histrap-hp-histidine-tagged-protein-purification-columns-p-00250>

LINK

5mL

SPECIFICATIONS

- 20.1 **Column preparation:** The column was first washed with 5 column volumes (25 mL) of water, followed by equilibration with 5 column volumes of binding buffer.
- 21 **Column loading:** A total of 4.2 mL of protein extract was injected into the column via the ÄKTA pure system.
- 22 **Column washing:** After sample injection, the column was washed with Buffer A using the ÄKTA pure system until the baseline stabilised. The cleaved protein, 3×FLAG-tagged *AtGa3ox1*, did not bind to the column and was recovered in the flow-through.
- 23 The column was washed with 4 column volumes of Buffer B.

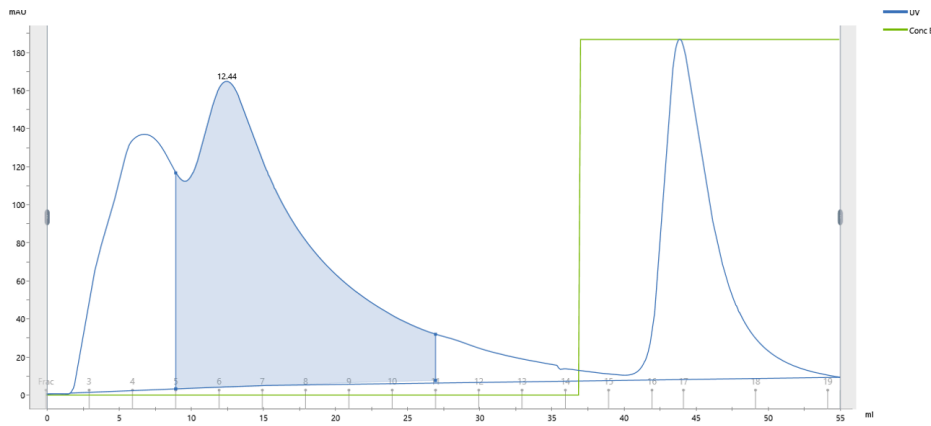


Figure 6. Purification of 3×FLAG-tagged *AtGa3ox1* using a HisTrap column (Cytiva). The reaction mixture containing 7×His-tagged TEV protease and the cleaved target protein was injected into the ÄKTA pure system at a flow rate of 5 mL/min. The column was washed with Buffer A (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 20 mM imidazole; 5% glycerol). The cleaved 3×FLAG-tagged *AtGa3ox1* did not bind to the column and was recovered in the flow-through. The column was then washed with Buffer B (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 500 mM imidazole; 5% glycerol) to elute remaining His-tagged components.

24 and Fractions 3 to 16 were analyzed by SDS-PAGE on a 10% polyacrylamide gel.

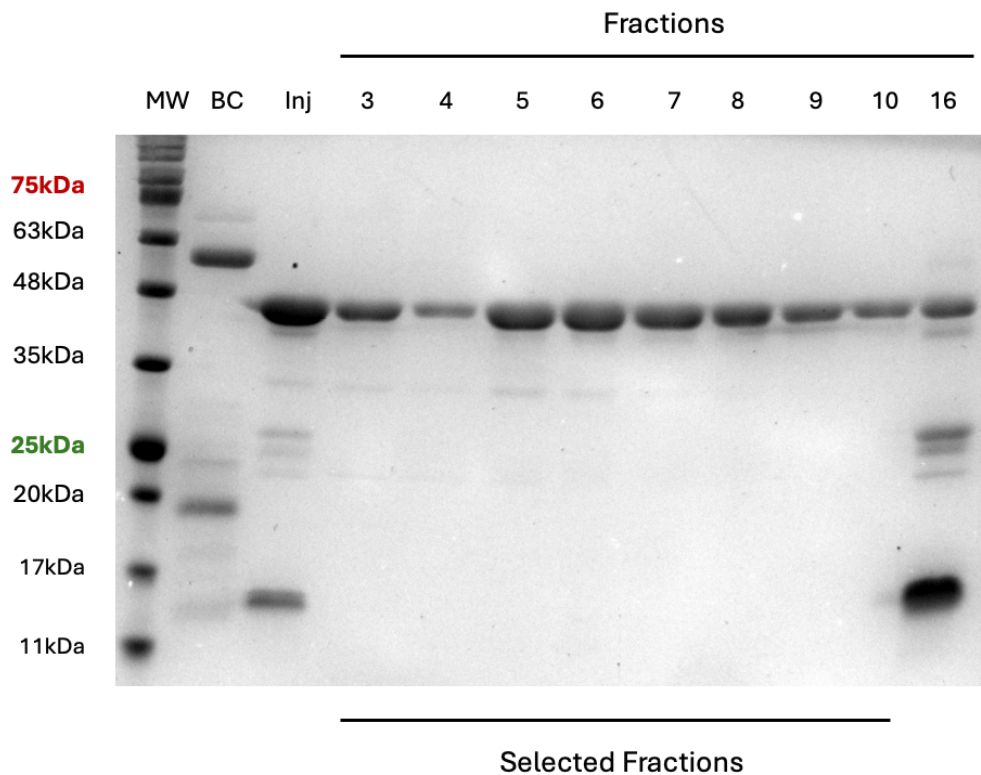


Figure 7. SDS–PAGE analysis of 3×FLAG-tagged *AtGa3ox1* eluted from a HisTrap column (Cytiva).

The reaction mixture containing 7×His-tagged TEV protease and cleaved protein was injected into the ÄKTA pure system (injected, Inj). A sample taken before TEV cleavage was also loaded (BC, before cleavage). The column was washed with Buffer A (20 mM potassium phosphate, pH 7.5; 250 mM NaCl; 20 mM imidazole; and 5% glycerol), and the cleaved 3×FLAG-tagged *AtGa3ox1* eluted in the flow-through. Fractions 3 to 16 were analysed by SDS–PAGE and stained with Coomassie Blue (Blue Safe, NZYTech). Molecular weight marker (MW) and corresponding sizes are indicated on the left. Fractions 5 to 10 were selected for further use.

- 25 Fractions 5 to 10 were pooled and concentrated using an Amicon Ultra-15 centrifugal filter unit (10 kDa cutoff). The sample was centrifuged at 4,000 × *g* at 4 °C in a swing-bucket benchtop centrifuge, in 10-minute increments, until the desired final volume was reached.

Note

Note: No precipitate was observed during the concentration process.

The concentrated protein solution was then loaded onto a PD-10 desalting column to exchange the buffer to storage buffer, Buffer E (20 mM KPi pH 7.5 and 100 mM NaCl).

- 26 To store the protein, the buffer was exchanged, using a PD-10 desalting column.
Column preparation: The PD-10 desalting column was equilibrated with a total of 25 mL of buffer E (20 mM KPi pH 7.5 and 100 mM NaCl).
- 26.1 **Column loading:** A total of 2.5 mL of the selected and concentrated fractions (from Step 25) was slowly and directly loaded onto the PD-10 column.
- 26.2 **Elution:** Proteins were eluted by centrifugation at $1,000 \times g$ for 2 minutes at 4 °C.

Note

Note: After elution, 20% glycerol was added to the proteins for long-term storage.

Mass Spectrometry (Optional step)

- 27 The identity of the purified protein was confirmed by LC-Mass Spectrometry.



Protocol references

- [1] Hedden, P., & Phillips, A. L. (2000). Gibberellin metabolism: new insights revealed by the genes. *Trends in plant science*, 5(12), 523–530.
- [2] Luís, I. M., & Abreu, I. A. (2025) Preparation of level 0 modules for Golden Gate assembly in pET28g, a new tool to prepare pET-based vectors for protein expression in *Escherichia coli*. ([dx.doi.org/10.17504/protocols.io.j8nlk9e75v5r/v1](https://doi.org/10.17504/protocols.io.j8nlk9e75v5r/v1))
- [3] Engler C, Youles M, Gruetzner R, Ehnert TM, Werner S, Jones JDG, et al. A Golden Gate modular cloning toolbox for plants. *ACS Synth Biol*. 2014;3: 839–843.

Acknowledgements

We gratefully acknowledge Cristina Timóteo, Rita Pacheco, Teresa Baptista da Silva, and the Protein Purification Facility at ITQB-NOVA for their invaluable assistance with bacterial cell disruption and affinity purification. Mass spectrometry data were generated by the Mass Spectrometry Unit (UniMS) at ITQB/iBET, Oeiras, Portugal.

We acknowledge the Portuguese Fundação para a Ciência e a Tecnologia (FCT) for a fellowship for JM (PD/BD/06917/2020, 10.54499/[2020.06917.BD](https://doi.org/10.54499/2020.06917.BD)). Our work was supported by the FCT research fund GREEN-it 'Bioresources4sustainability' (UIDB/04551/2020, <https://doi.org/10.54499/UIDB/04551/2020>, and UIDP/04551/2020, <https://doi.org/10.54499/UIDP/04551/2020>).