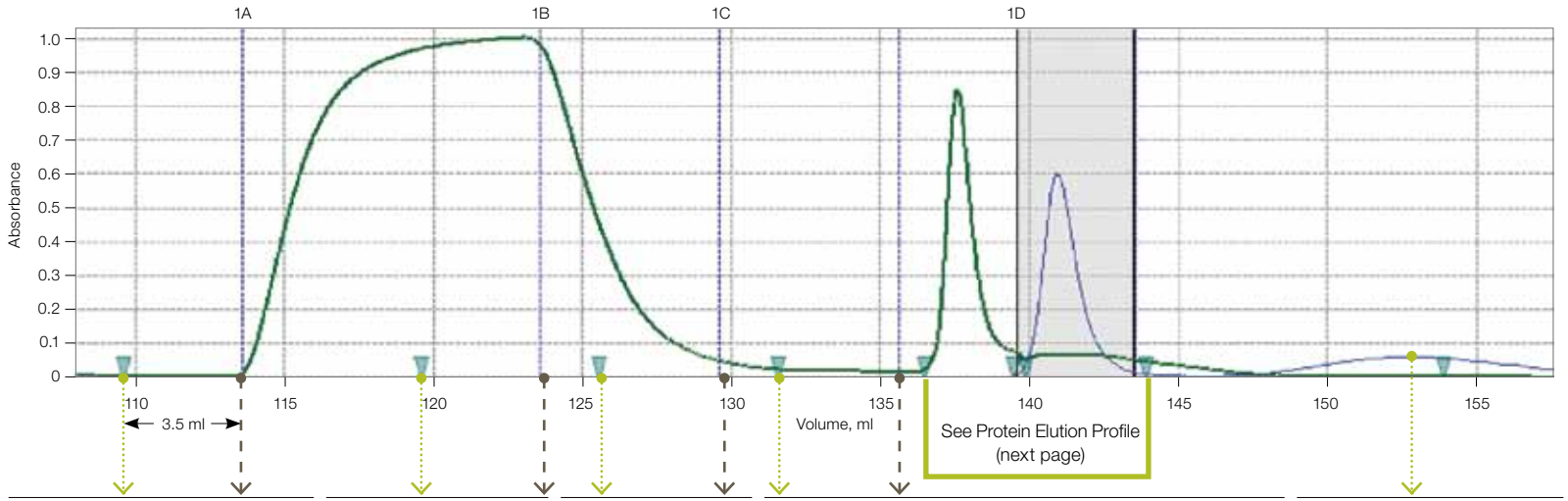
Understanding Chromatograms Generated by the Profinia Protein Purification System

Understanding Profinia system-generated chromatograms will help you analyze the protein purification procedure, identify problems, and fine-tune the purification procedure in programmed methods. This reference guide uses the IMAC + desalting purification method as an example to explain the steps carried out during the protein purification process.

Chromatogram for 1 ml IMAC + Desalting Method

Sample Loading, Wash Steps, and Protein Elution Profile



Sample loading step starts (10 ml)

- Flowthrough is collected in fraction 1A (sample #1)
- Flowthrough collection is delayed by ~3.5 ml. This accounts for the system volume between the buffer/ sample valve and the fraction collection valve

Wash-1 step starts (10 ml sample loading is complete)

- Wash-1 is collected in fraction 1B (sample #1)
- Collection to 1B is delayed to account for system volume
- Collection into 1A continues until 10 ml of flowthrough is collected. Collection then switches to 1B (Wash-1)

Wash-2 step starts (Wash-1 is complete)

- Wash-2 is collected in fraction 1C after Wash-1 collection into 1B is complete
- Collection to 1C is delayed to account for system volume

Elute-1 C1 step starts (Wash-2 is complete)

- Output for the Elute-1 step is sent to the waste when the Wash-2 collection into 1C is complete

  Note: During this step peak detection is activated (as indicated by a light bulb icon next to the UV line in the lower part of the display)

  If a peak is not detected within a specified volume (for 1 ml IMAC, this volume is 6.3 ml), the next step Elute-2 C1 will be activated
- Collection to waste at this step is also delayed to account for system volume

Imidazole peak eluting from the desalting cartridge

- Absorbance ~0.05–0.06

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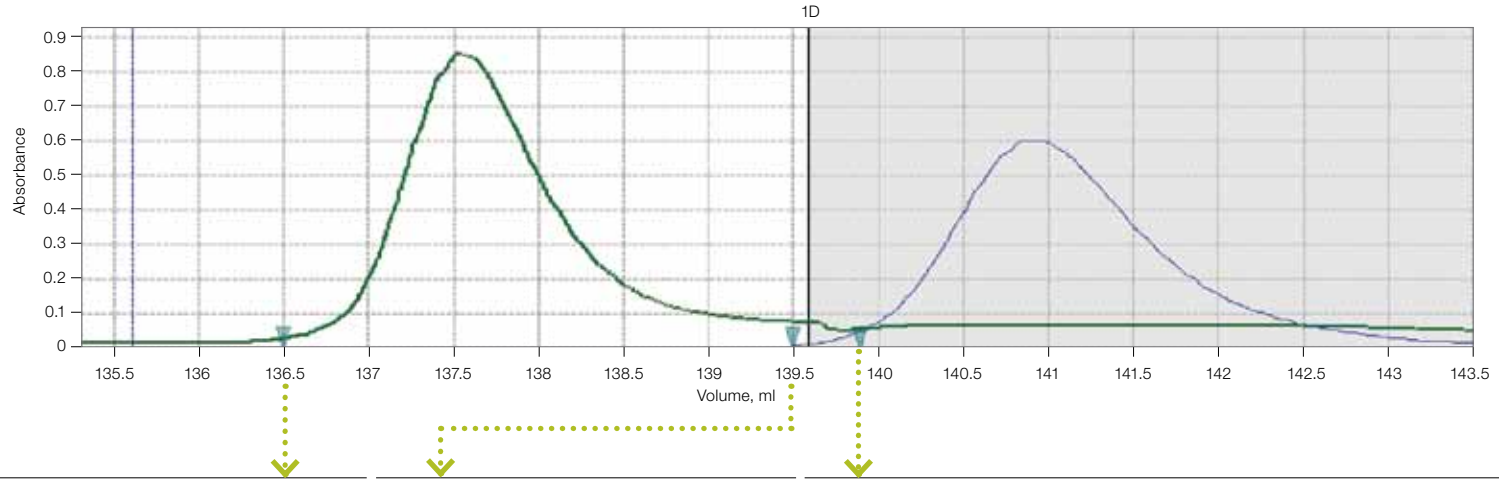
Protein Elution Profile — 1 ml IMAC + Desalting Method



Elute-2 C1 step starts

- The start of the affinity protein elution peak is detected at the end of the Elute-1 C1 step. During the Elute-2 C1 step a set volume (3 ml) is diverted to 10 ml desalting cartridge (C2) from 1 ml affinity cartridge. This volume can be edited in the Program Method mode. Volumes greater than 3 ml (for a 10 ml desalting cartridge) will exceed the desalting cartridge capacity and result in loss of protein and incomplete desalting

Elute-1 C2 step starts

- The 3 ml protein affinity peak has been loaded onto the desalting cartridge and the desalting step starts
- During the Elute-1 C2 step, automatic peak detection is activated (as indicated by a light bulb icon next to the UV line in the lower part of the display)
- If a peak is not detected within a specified volume (for 10 ml desalting cartridge, this volume is 1.5 ml), the next step Elute-2 C2 will be activated

Elute-2 C2 step starts

- The start of the protein desalting peak is detected at the end of the Elute-1 C2 step
- During the Elute-2 C2 step, a set volume (4 ml) of the purified and desalted protein is collected in Fraction 1D (sample #1). This volume can be edited in Program Method mode to a volume larger or smaller than 4 ml

  NOTE: The gray area of the chromatogram indicates what is actually collected in fraction 1D.

  When a peak is detected in UV2, there is a fraction valve switch delay to compensate for the system volume between UV2 and the fraction valve.

Note: Chromatogram is amplified from page 1.

Step Parameters for Elute-1 C1 (Affinity) and Elute-1 C2 (Desalting) in Program Method Mode

Affinity Protein Peak Elution

During the Elute-1 C1 step, the system starts pumping elution buffer and continues until the start of the affinity protein peak is detected. Then the system switches to Elute-2 step. The step parameters used below are the parameter values for the 1 ml affinity cartridge (C1) of the 1 ml IMAC + desalting method and are different for other methods. Some of the parameters can be edited in the Program Method mode.

Step Elute-1 C1		
Flow rate	2.00 ml/min	(Editable)
Peak detect delay	105 sec	(Editable)
Step time	3.1 min	
Max peak hold volume	6.3 ml	(Editable)
Concentration	B3-2X	(Editable)
Frac	W	

Peak Detect Delay: The automatic peak detection is activated after this delay. The default value is based on the system volume and is calculated from the flow rate value. When the flow rate is changed, the peak detect delay value is automatically adjusted.

Note: The peak detect delay can be edited in the Program Method mode. For example, if you want to manually detect the peak (available from the display during this step), you can enter a large value to ensure that the automatic peak detection is never activated.

Max Peak Hold Volume: This is the maximum volume the system will use before overriding automatic peak detection and proceeding to the next step. This value is intended to prevent or minimize loss of protein, in the event that the protein peak is not automatically detected, for example, in cases of very low expressed proteins or for proteins that do not absorb at 280 nm.

The default value is based on the instrument system volume — the volume between the buffer/sample valve and UV-1 detector.

Note: This value can be edited in the Program Method mode.

Desalt Protein Peak Elution

During the Elute-1 C2 step, the system starts pumping the desalting buffer and continues until the start of the desalted protein peak detection. Then the system switches to Elute-2 step.

Below are the parameter values for the 10 ml desalting cartridge (C2) of the 1 ml IMAC + desalting method.

Step Elute-1 C2		
Flow rate	2.00 ml/min	(Editable)
Peak detect delay	0 sec	(Editable)
Step time	0.8 min	
Max peak hold volume	1.5 ml	(Editable)
Concentration	B4-5X	(Editable)
Frac	W	

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