

Optimization of Chromatography System for use with CIMac™ Analytical Column

The main goal of this document is to advise how to shorten the flow path of (HP)LC system so that it can be used with a CIMac™ column.

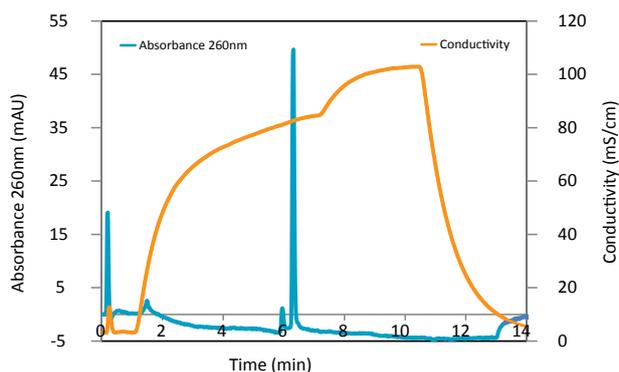
In order to do so, the following steps should be taken:

1. The smallest possible mixing chamber should be used. If the instrument is equipped with a large dead volume dynamic mixing chamber with a stir bar, replace it with a smaller mixing chamber (smaller than the volume of column). In case such equipment is not available, replace the existing mixing chamber with a plain tee piece.
2. Tubing that connects the mixing chamber to the detector cell should be replaced with smaller diameter tubing (e.g. green 0.75 mm I.D. tubing should be replaced with 0.25 mm I.D. blue tubing). Tubing should be kept as short as possible.
3. Adjust the averaging time of the UV monitor to a value around 0.1 (every 0.1 seconds, data from detectors are averaged and saved as one data point).
4. If possible, try to shorten paths between the CIMac™ column and UV detectors as well as the injection port. Circumvent multi-column valves by placing the CIMac™ column directly in line between the injection port and detectors to reduce dead volumes and ensure a direct signal. Set the maximum allowed pressure.
5. Disconnect all parts (e.g. in-line filter...) between the pump and the column to avoid any increase of axial dispersion and loss of resolution as a consequence!

Practical example:

A comparison on a regular and optimized ÄKTApurifier System was performed using a CIMac™ pDNA column under the same conditions with the same sample.

Regular system



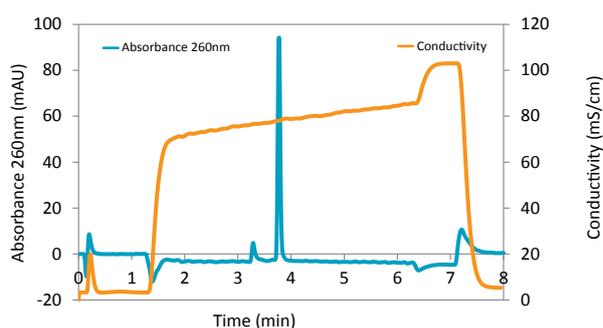
Conditions:

- Flow rate: 2 mL/min
- Sample: clarified plasmid pJ (5.6 kbp), concentration 36 µg/mL
- Column: CIMac™ pDNA (Prod. No.: 150.0001)
- Injection volume: 100 µL
- NaCl gradient: step up to 65% B; then linear gradient to 80% buffer B in 5 min (30 column volumes)

Buffers:

- A: 50 mM TRIS pH 8
- B: 50 mM TRIS, 1M NaCl pH 8

Optimized system



It takes twice as much time on a regular system to achieve the best possible separation of SC and OC pDNA forms as it does on an optimized system.

With improved optimization you can achieve higher sensitivity and better resolution.



For any additional information please contact us:

sales@biaseparations.com

Tel.: +386 5 9699 500

orders@monoliths.com

Fax.: +386 5 9699 599

tech-support@monoliths.com

www.biaseparations.com

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