Contichrom® Discovery
Automated Batch and Multi-Dimensional FPLC Protein Purification System
Availability of pure proteins in quantities of tens of milligrams is a prerequisite for many life science applications such as for assay development and structural studies.

The Contichrom Discovery is an easy-to-operate preparative FPLC protein purification system offering an expandable library of single-column batch and automated multi-step purification protocols for antibodies and tagged proteins.

For the latter, protein purification protocols are sequentially coupled and highly automated to add convenience, reproducibility and speed. Automated multi-step purification protocols are particularly useful for classes of proteins, such as antibodies and tagged proteins where a standard multi-step protocol can be established minimizing manual intervention.

**Discovery Wizard: One Process with up to Three Chromatography Steps**

- **Untagged Protein**
  - Single-column batch purification → Batch Wizard
  - Recombinant proteins

- **Tagged Protein**
  - Automated multi-step purification (mAbs, tagged proteins) → Discovery Wizard

- **Discovery Wizard**
  - Affinity capture
  - Polishing
  - Desalting/buffer exchange
  - Ready-to-use Protein
  - pure & formulated
  - >98% pure
  - >95% pure
  - expressed protein
  - Downstream step
The Contichrom Discovery system and its operating software ChromIQ offers batch functionality as well as automated multi-step processing functionality for purification of proteins and oligonucleotides. These include automated capture steps for monoclonal antibodies (mAbs) or tagged proteins, for example His-tags or Fc-tags, as well as orthogonal connected second polishing steps, for example size exclusion chromatography (SEC) or ion exchange chromatography (IEX) and further desalting / buffer exchange steps.

**The Batch wizard** functionality of the ChromIQ software provides easy design and operation of all single column processes with isocratic and gradient mode.

**The Discovery wizard** provides a library of automated 1-, 2- and 3-step purification recipes. Some suitable resin consumables are provided with the system as a purification kit.

**Customization:** you can customize purification steps and connect them to extend the given recipe library or to design new purification sequences from scratch.

**High Throughput:** with an additional Autosampler and Software upgrade you can get unattended high throughput purification capabilities of up to 94 samples with a 3-step purification protocol per week.
The Contichrom Discovery system has been designed for **speed**, **flexibility** and **convenience**.

**Speed** is provided through the unidirectional design of connected purification steps with in-line dilution, avoiding any holding steps in loops. Uniquely, the eluates from one process step are not stored in intermediate loops but the different process steps are fully integrated and streamlined providing an unparalleled fast processing.

**Flexibility** is provided through the possibility to either use fixed recipes or preparing customized purification trains using the Discovery Wizard and by extending the purification train even further using normal single-column batch chromatography with the Batch Wizard. With accessory valves and a software upgrade, automated high throughput purification of multiple samples can be performed.

**Convenience** is provided through a high level of automation, the user-friendliness of the ChromIQ software and the seamless interplay of the system, the recipes and the pre-packed columns/consumables.

Schematic overview of the setup of the Contichrom Discovery.
The **Contichrom Discovery** system with its automated purification protocols and pre-packed columns have been designed for having the choice of obtaining either fast processing (loading protocol 1) or high load (loading protocol 2). Fast processing is obtained by limiting the load quantity to 10 mg of protein. High load is obtained by doubling the loading quantity and reducing the flowrate.

The pre-optimized multi-step protocols all start with an affinity capture step, followed by desalting or a polishing step and a subsequent optional desalting step. Typically, 10-20 mg of protein can be recovered per run, depending on the molecular weight and whether a high throughput or a high yield protocol is chosen. Purification protocols and a choice of recommended pre-packed columns provide convenience and flexibility.

Different purification protocols and different sample loads require different process times, as shown in the table below.

<table>
<thead>
<tr>
<th>Multi-step protocols</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>IMAC</td>
<td>IMAC</td>
<td>Protein A</td>
<td>Protein A</td>
<td>Protein A</td>
<td>Protein A</td>
</tr>
<tr>
<td>Step 2</td>
<td>Size Exclusion</td>
<td>Desalting</td>
<td>Size Exclusion</td>
<td>Desalting</td>
<td>CIEX or AEX</td>
<td>CIEX or AEX</td>
</tr>
<tr>
<td>Step 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Desalting</td>
</tr>
<tr>
<td>Process time</td>
<td>118*</td>
<td>45*</td>
<td>102</td>
<td>27</td>
<td>47</td>
<td>68</td>
</tr>
<tr>
<td>min/sample for 10 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>min/sample for 20 mg</td>
<td>120</td>
<td>47</td>
<td>107</td>
<td>32</td>
<td>52</td>
<td>73</td>
</tr>
</tbody>
</table>

Loading protocol 1: consists of a loading speed of 600 cm/h and a loading volume of 10 mL.
Loading protocol 2: consists of a loading speed of 300 cm/h and a loading volume of 20 mL.
*Loading speed restricted to 300 cm/h.*
The ChromIQ operating software supports two different wizards enabling an intuitive, user-friendly operation for batch and automated multi-step connected batch processes.

The Batch Wizard helps to design single-column chromatography runs in both isocratic and gradient mode. The Discovery Wizard supports pre-defined automated recipes for one, two- or three step integrated chromatography. The recipe libraries of both Wizards can be extended with own, customized recipes and with additional recipes from ChromaCon.

ChromIQ includes a number of features that are particularly helpful for automated processes, such as a buffer management system showing the recipes for buffer preparation, the number of runs that can be performed with the remaining buffer volumes, and a pressure watchdog preventing the system from running dry.
The ChromIQ user interface for multi-dimensional purification is easy to use. It provides all necessary information where it is needed – and only when it is needed. The Discovery Wizard helps choosing the right pre-optimized process for typical purification challenges. During the run, the current status of the system and of the processes are displayed.

An animated flow-sheet provides real-time information on the system status and process. The buffer management status (displayed on the left) shows remaining buffer volumes in real-time.

Discovery Wizard for 3-step purification. Pre-optimized protocols can be chosen. Basic information on the processes is displayed directly inside the software (displayed on the right).
1. The entry page shows options for 1-, 2- or 3-step purifications and a button to prepare the system for storage. Click the “2-step Purification” button, which will turn blue.

2. The middle column shows the different purification sequence options and also the possibility to run custom 2-step processes. Click the “IMAC-SEC” button which will turn blue. Now general information on IMAC/SEC processes is displayed on the right. Next, press “START 2-step”.

3. After entering the project and sample names, this screen with information for getting the system ready to run is displayed. The picture shows where to mount which column. The “Mount Column” button shows detailed information and allows to flush the column with buffer for pre-equilibration.
Walkthrough Example: 2-step IMAC-SEC

4. The right table shows the required buffers for the selected purification method. Current and required buffer volumes are displayed. The table on the bottom left shows the connection between the color-coded buffer inlet tubes and the buffer tanks.

5. The software reminds you once more of the critical steps for preparing the purification run.

6. Finally, the software guides through the process of purging the pumps with buffers and samples. Afterwards, you can “Start the Run” to automatically purify your protein.
Application Examples

2-step purification of a monoclonal antibody

Protein A / Desalting

**Conditions**

Feed: 10 mL clarified cell culture supernatant (monoclonal antibody), 1 mg/mL
Step 1: Protein A (Affinity capture)
Step 2: Desalting (Buffer exchange)

**Run**

- **Step 1**: Protein A (Affinity capture)
  - Load
  - Equilibration
  - Wash
  - Elution
  - Transfer to desalting column

- **Step 2**: Desalting (Buffer exchange)
  - Elution buffer
  - Final product
  - Standard Protein A affinity capture step
  - Final sample is buffer exchanged and ready to use

**Results**

- **SDS-PAGE**
  - Marker
  - Feed
  - Product
  - Purified mAb

- **SE-HPLC**
  - 96.0% monomer
Application Examples

2-step purification of a His-tagged protein

**IMAC / Desalting**

**Conditions**

- **Feed:** 5 mL, E. coli BL21(DE3) cell lysate, 0.5 mg/mL
- **Step 1:** IMAC (Affinity capture)
- **Step 2:** Desalting (Buffer exchange)

**Run**

![Chromatogram](chart.png)

**Results**

![SDS-PAGE](chart2.png)

**SE-HPLC**

98.1% monomer
### Application Examples

#### 3-step purification of a monoclonal antibody

**Protein A / CIEX / Desalting**

### Conditions

**Feed:** 10 mL clarified cell culture supernatant (monoclonal antibody), 1 mg/mL  
**Step 1:** Protein A (Affinity capture)  
**Step 2:** CIEX (Polishing)  
**Step 3:** Desalting (Buffer exchange)

### Run

- **Step 1:** Equilibration  
- **Step 2:** Protein A Elution, Transfer to CIEX column, Salt Gradient  
- **Step 3:** Final product

- **Standard Protein A affinity capture step**
- **Integrated CIEX aggregate removal step**
- **Final sample is buffer exchanged and ready to use**

### Results

- **SDS-PAGE**
  - Marker, Feed, Product, Purified mAb
  - MW [kDa]: 250, 150, 75, 50, 37, 25, 20, 15, 10

- **SE-HPLC**
  - 3-step protocol = 0.5% dimer  
  - 2-step protocol without CIEX = 4% dimer
The **Contichrom Discovery** HT system with an autosampler can be used for unattended, high-throughput purification of monoclonal antibodies or other protein samples using a multi-step purification protocol with up to 18 sample loads. The purification protocols are integrated without intermediate loop storage of eluates, thus providing short processing times for multi-step purification protocols. If the second process step requires a different buffer composition than the eluent of the first step, inline-dilution can be used.

High throughput purification application are particularly useful for screening of monoclonal antibody variants from different clonal expression variants or for screening of protein mutants.

Chromatogram of an unattended and automated 3-step purification of a 10 mL monoclonal antibody (mAb) feed volume – Titer of 1 mg/mL. Total run time: 68 min including cleaning.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Number of purified mAbs</th>
<th>System run time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working day</td>
<td>21</td>
<td>24 h</td>
</tr>
<tr>
<td>Week (24/7)</td>
<td>147</td>
<td>166 h</td>
</tr>
</tbody>
</table>

Up to 147 protein samples can be purified per week with a 3-step purification protocol with 10 mg of protein obtained per run.
System Accessories

External variable multi-wavelength detector (190-700nm)

Cooling chamber with Foxy R-1 fraction collector

Sample loading loop kit 500 µL up to 20 mL

External variable single wavelength detector (190-500nm)

For details about more additional accessories please ask your local ChromaCon representative.
High Throughput

The Discovery System can be upgraded to high throughput (HT) mode being able to process unattended up to 94 samples per week with a 3-step purification protocol for mAbs including a Protein A capture, a CIEX gradient polish step and a desalting step. The HT system consists of a CETAC ASX-560 autosampler, the Discovery system and a fraction collector. The whole system setup including the autosampler can be placed in a cold room or optional cooling chamber.

### Throughput comparison for different methods:

<table>
<thead>
<tr>
<th>Step #</th>
<th>Protocol scheme</th>
<th>Single purification time (min)</th>
<th>Number of samples processed in 3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-step basic</td>
<td>Protein A</td>
<td>12.5</td>
<td>344</td>
</tr>
<tr>
<td>2-step desalting</td>
<td>Protein A - desalting</td>
<td>27</td>
<td>160</td>
</tr>
<tr>
<td>2-step polishing</td>
<td>Protein A - SEC</td>
<td>102</td>
<td>42</td>
</tr>
<tr>
<td>3-step polishing</td>
<td>Protein A – CIEX - desalting</td>
<td>68</td>
<td>63</td>
</tr>
</tbody>
</table>

- Assuming 3 days of continuous processing using the autosampler
- Assuming 10 mL load per sample
- Data generated using method templates and columns supplied with the system and recommended for optimal performance
Bio/Pharmaceutical companies
- High-throughput purification of mAbs and tagged proteins with a SEC polish step
- Conventional batch purification of proteins
- Process development for therapeutic proteins

Research institutions
- Biomarker discovery, target identification, protein-based assays
- Fast production of biological targets without need for manual optimization of purification protocols

CDMOs and CROs producing Biologics
- Process development of Biologics
- Production of recombinant proteins for pre-clinical research

Universities and colleges
- Protein purification for non-protein experts
- Unlock new research opportunities as high quality protein targets become affordable and easily accessible
- Production of biological targets using tagged proteins
- Protein purification for protein crystallographers

Who uses the Contichrom Discovery?
3rd dimension desalting
1st dimension separation
2nd dimension separation
Installation & After Sales Services

All systems are pre-tested to verify the high level of quality. The system is delivered pre-assembled. Installation is done with an automated installation qualification and operation qualification (IQ-OQ) protocol that re-checks the system and generates a test summary. The Contichrom Discovery is then ready for use; the Discovery kit contains a full set of pre-packed columns suitable for the multi-step purification method recipes.

We offer on-site and off-site training, webinar-based product support and we organize workshops on continuous chromatographic purification. Finally, you can always call us or our local partners for troubleshooting.

Purchasing an FPLC System and operating it is only part of a customer's value proposition.

After sales support such as Preventive Maintenance (PM) and total life cycle costs are an important consideration in a system’s procurement evaluation. We offer PM, repair and system validation and qualification support including IQ-OQ and training. We also offer free ChromIQ software upgrades.

Our system is designed to have very low maintenance costs: only wear parts from pumps and valves need to be exchanged occasionally in an easy way without disassembling the system. The UV detectors have a long lifetime and lamp changes are feasible. We recommend to exchange the wear & tear parts every 6 months (heavy use) or every 12 months (regular use). We provide instructions how to do this but you are also welcome to get help from our PM partners.

We offer comprehensive and cost-effective Preventive Maintenance and Repair Service packages.

Worldwide Preventive Maintenance and Repair Service packages. On-site and off-site service with fast turnaround times.

For details please ask for a quote at your local ChromaCon representative.
Contichrom® Discovery Functions

- 2 UV and conductivity cells
- pH electrode
- Outlet valves
- Inlet valves
- 2 Buffer selection valves
- 16 total inlets
- Manual purge valves
- Injection & drain valve with injection loop
- 2 Buffer selection valves
- 2 Pressure sensors
- System/gradient pumps
- Static mixer
- External valve (column selection, up to 4 columns)
- Buffer tray
## Technical Specifications

<table>
<thead>
<tr>
<th>Process capabilities</th>
<th>Batch Wizard (isocratic, step, gradient elution), Discovery Wizard for automated 2- or 3-step purification. Upgradable for high-throughput purification with additional external valves for sample loading.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operating software</td>
<td>ChromIQ is a user-friendly operating software with step-by-step wizards to guide the design and automatically run single column and multi-dimensional batch chromatography runs.</td>
</tr>
</tbody>
</table>
| Software compliance  | The software contains essential elements of 21CFR Part 11 compliance:  
  - Pre-defined user groups, administrators, R&D and production users  
  - Rights management for individual user groups  
  - User accounts are password protected  
  - Logging with time stamp and user name (non-deletable)  
  - Electronic signature with checksum of log and measurement files |
| Pump type            | 2 High precision double-piston pumps with active seal wash |
| Pressure             | Rating: 50 bar (5 MPa) / 725 psi  
  Sensors: 2, after each pump |
| Flow rate range      | 0.1 – 36 mL/min |
| Buffer selection     | 16 Inlets (2 x 8-fold buffer selection valve)  
  2 Outlets |
| UV, fixed wavelength | 2 Long lifetime LED UV detectors, each with 254 & 280 nm recorded simultaneously |
| Conductivity monitoring | 2 Conductivity sensors (1-300 mS/cm) |
| pH monitoring        | 1-14 |
| Valves               | 6 Multi-position valves  
  1 Automated drain & injection valve  
  1 External column selection valve |
| Mixer                | Static mixer with non-return valve  
  0.1-36 mL/min |
| Sample loading       | Sample pump for drawing samples from bottles or vials. Multiple automatic injections possible. Injection valve for injection loop loading. Injection loops (500 µL-20 mL) for syringe injections available as accessories. High-throughput option with autosampler |
| Buffer management    | Monitored buffer tank levels. Pressure drop watchdog to prevent system from running dry. |
| Fraction collection  | FOXY R1 as standard – supporting multiple rack types |
| Computer hardware    | Stand-alone laptop computer (Windows, 64 bit, full HD resolution (1920 x 1080) or higher) with ChromIQ software |
| Other                | Cold room compatible  
  Large buffer tray  
  Portable & compact  
  Runs resins and membrane stationary phases. A starter kit of suitable pre-packed columns are included. |
| Dimensions           | 515 mm x 450 mm x 380 mm (20.3” x 17.7” x 15.0”) |
| Weight               | 30 kg (67 lb) |
| Materials            | All wetted parts are biocompatible  
  High pressure side capillaries: PEEK  
  Low pressure side tubing: PET  
  Fittings: PEEK |
| Upgrade capabilities  | High-throughput version with additional external valves or autosampler for sample loading |
Contact us now to find out how you can solve your separation challenges more easily!

Your contact at ChromaCon:

E-mail: info@chromacon.com

Web: www.chromacon.com

Your local representative:

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