

# PATfix<sup>®</sup> pDNA Platform

## Rapid At-Line Analytical Chromatography Solution

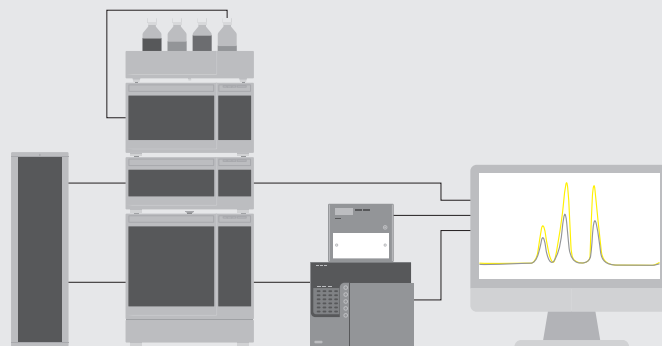
### Platform Overview

Global demand for pDNA production is at an all-time high. As an enabling product, pDNA is critical in production of mRNA, AAV and other therapeutic vectors. Increasing yield and purity in the production of pDNA is a vital step in meeting such demand. Supporting rapid process development and optimization, the PATfix<sup>®</sup> pDNA analytical platform provides reliable in-process control for pDNA process development and production.

PATfix<sup>®</sup> pDNA is a high-performance HPLC system, engineered for pDNA isoform and multimer separation using the unique analytical capability of the CIMac<sup>™</sup> pDNA monolithic column. User guides, validated methods, SOPs and pDNA standards are included. User-friendly PATfix<sup>®</sup> software makes for easy data processing, visualization and sharing.

### Key Benefits

- Fast, high resolution HPLC analytics for pDNA processing
- At-line monitoring for accelerated PD and secured production and quality
- More, key information with less time, labor and cost
- Unique CIMac<sup>™</sup> pDNA analytical monolithic columns
- User guides, specific validated methods, SOPs and standards included

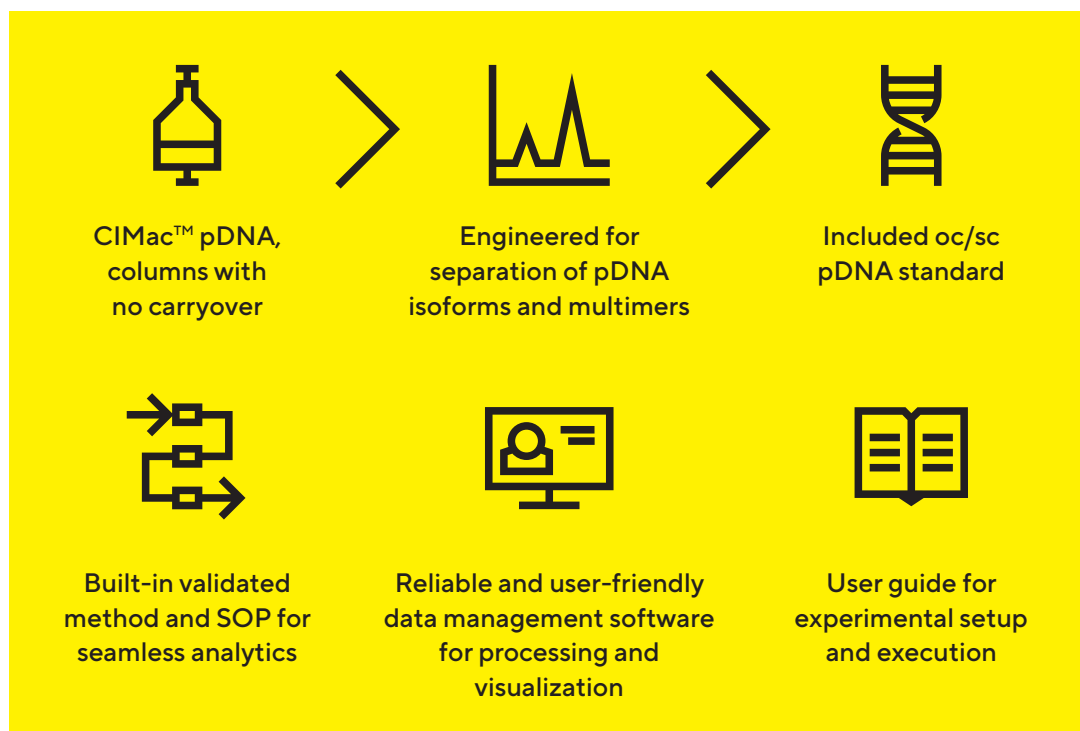


# PATfix<sup>®</sup> pDNA Platform

## Platform Description

PATfix<sup>®</sup> pDNA is the first member of the PATfix<sup>®</sup> product family. The portfolio will grow to include other platform solutions for mRNA and AAV, for example, and also various hardware solutions for analytical, semi-preparative and hybrid applications.

Key features of the PATfix<sup>®</sup> platform that support the analytical pDNA workflow are:



The PATfix® pDNA platform has four components:

- **PATfix® software**
- **the methods**
- **CIMac™ columns and**
- **the system hardware**

### **PATfix® Software**

An integral part of the PATfix® pDNA value proposition, the PATfix® software strips away the complexity of chromatography at the day-to-day operational level, while retaining it for higher level tasks.

- Single database of chromatograms is combined from multiple analytical systems
- Easily share interactive results with colleagues, customers and | or regulators
- Information extraction handled via user-defined templates
- Report generation helps ease the paperwork load
- Data visualization allows for quick progress during process development
- CFR Part 11 compliance allows easy implementation of PATfix® analytics in FDA approval process

### **PATfix® pDNA Methods**

Optimized analytical methods are a key component of a well-functioning analytical system. The PATfix® pDNA analytics platform includes fully optimized and validated analytical methods, as well as guidelines for buffer and sample preparation, including detailed SOPs. In addition, the PATfix® pDNA analytical package includes a pDNA calibration standard, which enables accurate quantification of the pDNA species of interest as well as batch-to-batch and day-to-day performance tracking. Additional in-process robustness is guaranteed by the conductivity and pH monitors, that ensure the analysis is being performed with correct buffers and gradients.

### **CIMac™ pDNA Column**

The CIMac™ pDNA 0.3 mL Analytical Column is built for fast and reproducible HPLC monitoring and quantitation of plasmid DNA. It separates different plasmid DNA isoforms, enabling in-process control and release testing of pDNA samples at all stages of the purification process. It can be operated at high volumetric flow rates (1 mL/min recommended); therefore, the information about the pDNA quantity and purity can be obtained quickly, reducing hold times. The CIMac pDNA column is a fast and reliable PAT tool. The 1.4 µm channel size version is appropriate for plasmids up to 8 KBp, while longer plasmids are best analyzed using the CIMac pDNA column with 6 µm channel sizes.

### **PATfix® System Hardware**

Achieving the correct hardware setup for reliable and reproducible analytical gradient separation of large biomolecules is not trivial, especially from complex mixtures and with pDNA samples frequently varying only in isoform | conformation.

To realize the required analyses, the PATfix® pDNA platform includes the following hardware:

#### **Pump**

The low pressure gradient pump with integrated degasser and mixer has bio-inert ceramic pump heads. Quaternary buffer switching allows workflows with 4 buffers and easy CIP implementation.

#### **Conductivity | pH monitor**

Contactless probe with a wide measuring range allows in-process monitoring of salt concentration and tracking of challenging methods that include pH gradients.

#### **Autosampler**

The autosampler accommodates vials or microtiter plates. Automated needle wash ensures minimal carry-over while temperature control of the sample tray secures sample stability.

#### **Column oven**

Controlled operational column temperature ensures optimal operation and maximal accuracy for analytical HPLC runs. The optional column oven accommodates up to 8 columns and manages temperature from 5 to 85 °C.

#### **Multiwavelength uv detector**

Highly sensitive monitoring of up to 4 wavelengths in the 190–700 nm range is possible, with intelligent temperature control minimizing drift.

#### **Fluorescence detector**

For tasks where additional characterization is required, e.g. challenging nucleic acid samples. The optional fluorescence detector is particularly useful for residual HCP and chromatin detection via native Trp fluorescence.

# Relevant Applications

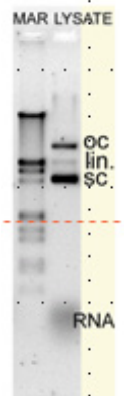
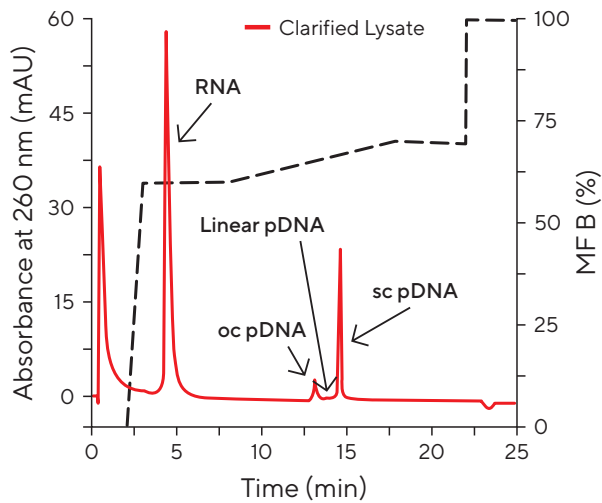
The PATfix® pDNA platform provides valuable analytical insights at all stages of pDNA processing, from upstream production through downstream purification and from process development to production of materials for clinical or commercial applications. At-line analytics support informed, accelerated process development and secure production runs and product quality.

## Use PATfix® pDNA for:

- Separation of pDNA isoforms and multimers
- Detection of process impurities, e.g. RNA, endotoxins, gDNA
- Quantification and purity analysis of pDNA species
- pDNA process development, e.g. lysis condition optimization
- In-process control of pDNA production at all process stages
- Batch-to-batch production and purification performance tracking
- Monitoring of pDNA linearization for mRNA production
- Product release and QA testing

## Applications Examples pDNA Production

### Separation of Isoforms and Detection of Process Impurities

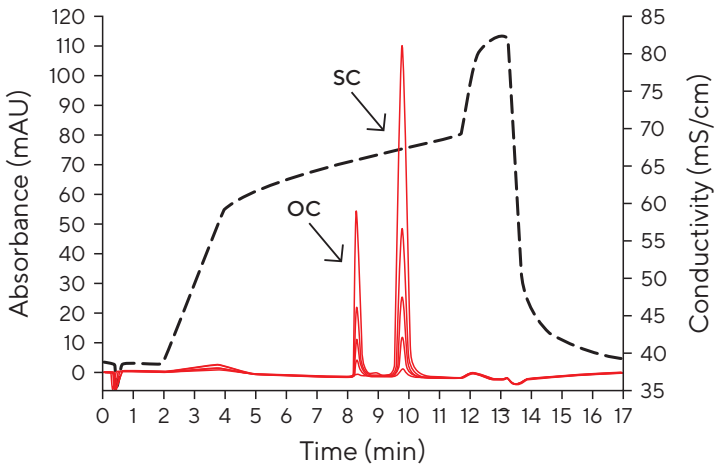


# Applications Examples pDNA Production

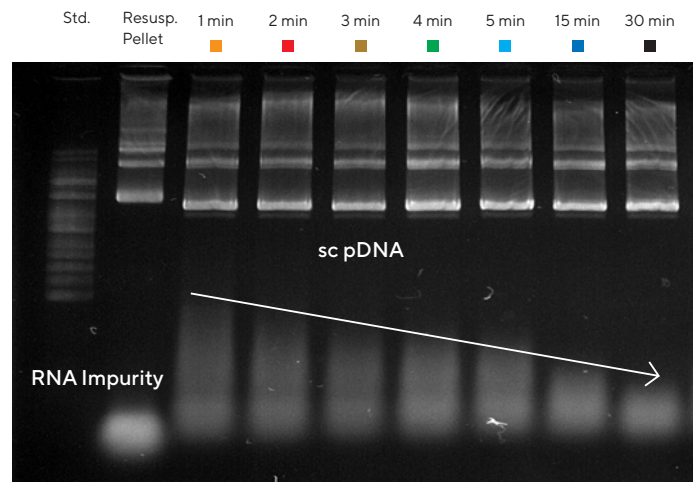
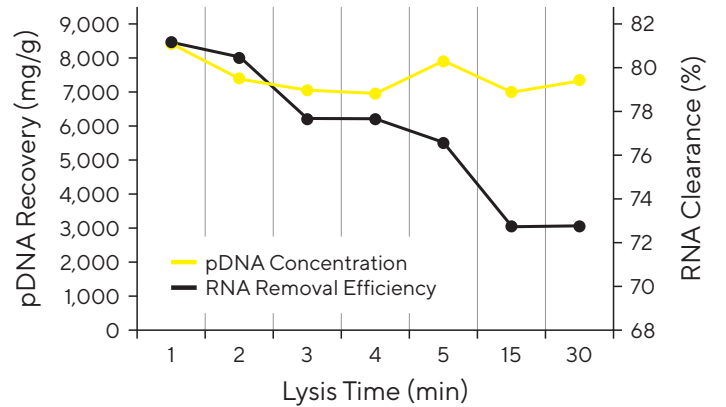
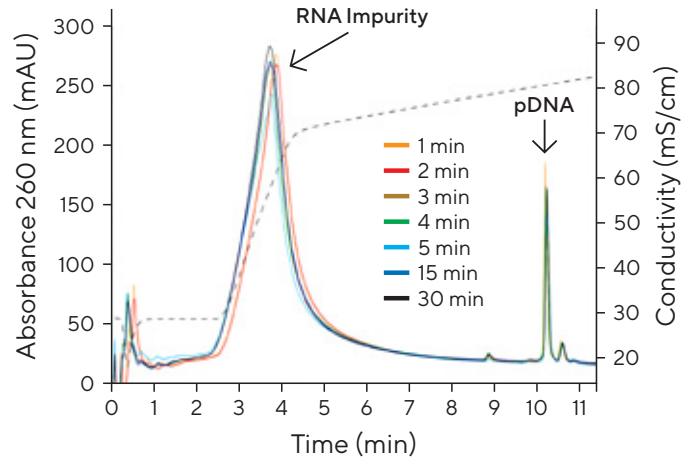
## Quantification

Column	CIMac™ pDNA Analytics Column
Samples	Different concentrations of pDNA standard 1, 5, 10, 20, 50 µg/mL
Detection	UV at 260 nm, Conductivity

Included pDNA calibration standard for quantification of oc and sc isoforms



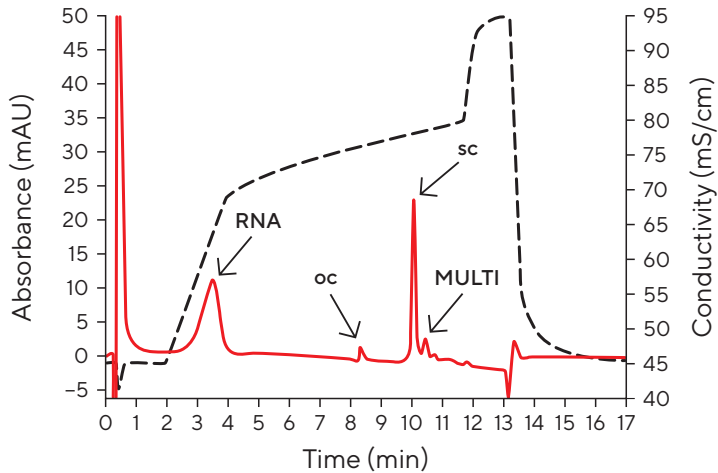
## Optimizing Lysis Conditions



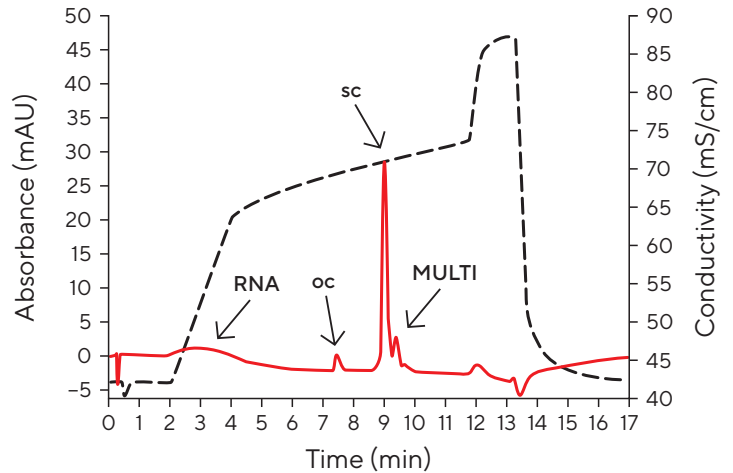
## pDNA Production: Isoform Separation and In-Process Control of Linearization

- CIMac™ pDNA allows monitoring of pDNA linearization progression and impurities
- Separation of many pDNA conformations (oc/sc/lin/multi), allowing for product quality control

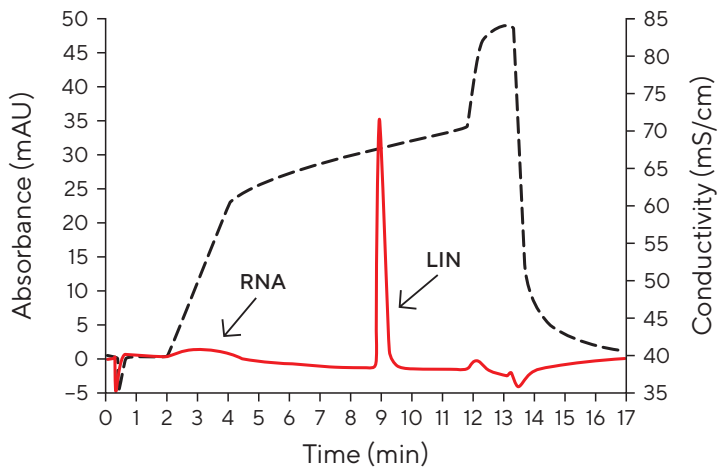
### 1. Clarified Lysate



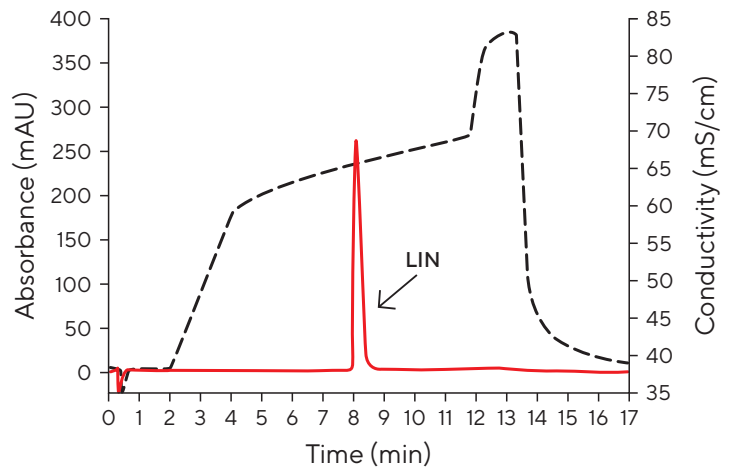
### 2. Capture Step Elution



### 3. Linear pDNA Before Polish



### 4. Linear pDNA After Polish



## Process Analytical Development Services

Extending the capabilities of the PATfix pDNA Platform, our process development scientists can develop a customized PATfix® pDNA platform, where the process analytical method is specifically optimized for your sample. Decades of experience working on pDNA has helped us acquire a wealth of knowledge, allowing us to complete process analytical development services for our clients' most challenging samples.

Besides method development and | or method validation of your sample, we will optimize the PATfix® pDNA platform with the protocols that best work for your sample, giving you a unique experience.



# Technical Specifications

## Pump

Quaternary low-pressure gradient pump	
Delivery system	Dual-piston pump
Pulsation compensation	Active pressure and pulsation compensation
Pulsation	< 2% Amplitude (typically: < 1,3%) or 3 bar (0,3 MPa), whatever is greater, at 1 mL/min ethanol, at all pressures > 10 bar (1 MPa, 147 psi)
Flow rate range	0,001–10 mL/min 0,1–8 mL/min (recommended)
Flow rate increment	0.001 mL/min
Flow rate accuracy	< 1% (measured at 5–80% of flow range, using ethanol)
Flow rate precision	≤ 0.1% RSD (based on the retention time at constant room temperature)
Maximum pressure	400 bar (40 MPa, 5800 psi)
Flushing piston seal	Standard
System protection	Soft start, Pmin und Pmax programmable
Wetted materials	Stainless steel, carbon-fiber-reinforced PTFE, FKM, PEEK, sapphire, ruby, aluminum oxide (Al <sub>2</sub> O <sub>3</sub> ), zirconium oxide

### Degasser module

Degasser channels	4 channels, Teflon® AF
Degasser max. flow rate	10 mL/min
Degasser method	Gas permeation using Teflon® AF amorphous fluoropolymer membrane
Degasser efficiency	< 0.5 ppm dissolved O <sub>2</sub> at 1 mL/min
Degassing chamber volume	480 µL volume per channel
Eluents	Limitations: Concentrated acids (1 mol/L or greater), hexafluoroisopropanol, sodium azide, fluorinated and perfluorinated eluents and freons
Wetted materials	PEEK, Tefzel®, Teflon® AF
Vacuum chamber	Polypropylene and stainless steel
Vacuum pump	Low hysteresis

Communication	
Interfaces	LAN, Pin header connectors (Analog IN, Start IN, Error IN)
Control	LAN, Analog and event control, Mobile Control
Analog input	0–10 V
Analog control input	Flow rate
Level   event outputs	8 event outputs (TTL, OC, Relais) and 24 V
Programming	19 programs, 9 program links, 1 WAKE UP program
GLP	RFID pump head detection, detailed report
Display	3 LEDs
Leak sensor	Yes
Protection type	IP-20

### Quaternary low-pressure gradient formation

Gradient type	Low-pressure gradient
Gradient range	0–100% 1–99% (recommended)
Minimum increment	0.1%
Gradient precision	±0.3% (measured at 1 mL/min, 150 bar, tracer: ethanol/caffeine) ±2% (1–99%, measured at 5–50% of the flow range, tracer: water/caffeine)
Gradient repeat accuracy	< 0.1% RSD (measured at 1 mL/min, 0.5% RSD overall, based on retention time at constant room temperature)
Mixing volume	250 µL (metal-free)
Delay volume	410 µL (metal-free)

## Autosampler

Max. plate   vial height	47 mm (incl. septa or capmat)
Sample capacity	Max. 768 samples (microtiter plates) or 108 standard autosampler vials
Injection volume range	0.1–10,000 µL programmable
Sample loop	100 µL
Dispenser syringe	250 µL
Headspace pressure	Built-in compressor, only for sample vials with septum
Switching time inj. valve	< 100 ms
Piercing needle precision	±0.6 mm
Sample tray cooling	With cooling function 4–40 °C
Vial detection	Missing vial/well plate detection by sensor
Needle wash	Programmable: wash between injections and wash between vial
Wetted materials	ETFE (buffer & needle tubing), silcosteel (sample needle, ), PEEK (valve stator, rotor seal), Kel-F (syringe valve), glass (syringe), PTFE (tip of syringe plunger)
Injection modes	Full loop filling, partial loop filling and microliter pickup, PASATM (pressure assisted sample aspiration)
Injection precision	RSD (Relative Standard Deviation): full loop filling < 0.3% partial loop filling at injection volumes > 5 µL: < 0.5% microliter pickup at injection volumes > 5 µL: < 1.0%
Injection volumes	full loop filling: max. 10,000 µL partial loop filling: 5,000 µL (50% of loop volume) microliter pickup: max. 4,625 µL (50% loop volume - 1.5 × needle volume) 0.1 µL increment for all injection modes
Sample carryover	< 0.05% with needle washing
Injections per vial	Max. 9 injections
Injection cycle time	Min. 7 s from the same vial, 14 s from different vials; < 60 s for > 100 µL sample injection in all injection modes, incl. 300 µL needle wash
Analysis time	Max. 9 h, 59 min, 59 s

### Communication

Interfaces	LAN, ANALOG
Control	Ethernet (LAN)
Inputs	2 programmable TTL inputs (next injection, freeze, stop)
Outputs	1 programmable relay output (inject marker, auxiliary, alarm)

## Conductivity | pH Monitor

Conductivity range	0.1–999 mS/cm
Conductivity accuracy	<5 % full scale end value
Temperature accuracy	±1.0 °C
Precision in measured range (0.1–300 mS/cm)	< 2% of end value or ≤ 5 mS/cm of higher values
Linearity	±1% scale end value
pH measured range	pH 2–12
pH precision	±0.2 pH in temperature range 4–25 °C
pH accuracy	±0.5 pH in temperature range 4–25 °C
pH drift	maximum 0.02 pH/h at pH 4
Maximum data rate	5 Hz (LAN, RS-232, Analog)
Outputs	LAN, RS-232, Analog
Analog output	conductivity, pH
Control	manual: front panel
Protection type	IP 20
Temperature range	4–40 °C; 39.2–104 °F
Air humidity	below 90%, non-condensing
Air pressure	84–106 kPa; 840–1060 mbar

### pH Measuring Kit

Maximum flow rate	80 mL/min
Delay volume	80 µL

### Conductivity Flow Cell, Analytical

Biocompatible	Yes
Fiber optics version	No
Capillary connection	¼"
Wetted materials	PEEK
Flow cell volume	30 µL
Maximum flow rate	10 mL/min
Maximum pressure	160 bar



## Multiwavelength UV Detector

Detection channels	8 (digital), 4 (analog)
Light source	Deuterium (D2) lamp with integrated GLP chip
Wavelength range	190 – 700 nm
Spectral bandwidth	< 8 nm at H $\alpha$ line (FWHM) Note: digital bandwidth 1 – 32 nm
Wavelength accuracy	$\pm 1$ nm
Wavelength precision	0.1 nm
Wavelength verification	Internal holmium filter and deuterium lines
Noise	$\pm 5$ $\mu$ AU at 254 nm
Drift	400 $\mu$ AU/h at 254 nm
Linearity	> 1.6 AU at 274 nm, typically 2.5 AU
Time constants	0.0   0.1   0.2   0.5   1.0   2.0   5.0   10.0 s
Integration time	Automatic (5 – 1000 ms)

### Communication

Interfaces	LAN (RJ-45), RS-232 (SUB-D 9), multi-pin connector, analog (RCA cinch connector)
Control	Front panel, Mobile Control, software, event control, analog, terminal protocol
Inputs	Error (IN), Start (IN), Autozero, Event 1–2
Outputs	Error (OUT), +5 V, Valve +24 V, Valve (OUT), Start (OUT)
Analog outputs	1 $\times$ 0 – 5 V scalable, 20 bit, offset adjustable

### Technical parameters

GLP	Detailed report including lamp recognition, operating hours, lamp operating hours, number of lamp ignitions, leak sensor
Display	Mobile Control (optional)
Ambient conditions	Temperature range 4 – 40 °C, 39.2 – 104°F, humidity: below 90%

### Analytical Pressure-Proof Flow Cell Cartridge

Path length	10 mm
Connection	1/8"
Volume	10 $\mu$ L
Wetted parts	Titanium, quartz, PEEK
Max. flow rate	20 mL/min
Max. pressure	300 bar

## Interface Box

### Analog Inputs (AN.IN)

Voltage range	2.56 to +2.56 V (bipolar mode)
Absolute maximum ratings	10 V to +10 V
Input impedance	10 M $\Omega$
Maximum resolution	24 bit
Minimum noise level	7 $\mu$ V (1 Hz, time constant 0.1 s)
Maximum data rate	up to 10 Hz (each channel)
Gain factor (for all channels)	1, 2, 4, 8, 16

### Analog Outputs (AN.OUT)

Voltage range	0 V to +10 V
Minimum voltage step (DAC resolution)	2.5 mV (12 bit)
Maximum load resistance	2 k $\Omega$

### Digital Inputs (DIG.IN)

Max. high level input voltage	+15 V
Min. high level input voltage	+2.5 V
Max. low level input voltage	+1 V
Min. low level input voltage	-15 V
Max. input, current at V(in) = 0,5V	10 mA

### Digital Outputs (DIG.OUT)

Output type	either NO or NC
Max. switching voltage	max. 175 V, DC
Max. switching current	max. 0.25 A

## Column Oven

Thermostating	
Temperature range	5–85 °C
Heating   Cooling range	2 °C/min
Temperature accuracy	±0.2 °C
Temperature stability	±0.1 °C

Communication	
Interfaces	LAN interface
Control	Optional for stand-alone functionality: Mobile Control

Column compartment				
Column dimensions	max. number	max. length*	max. outer diameter*	matching column
Heating   Cooling range	8	160 mm	12 mm	125 mm × 4.6 mm ID with precolumn
Temperature accuracy	4	325 mm	12 mm	300 mm × 4.6 mm ID
Temperature stability	1	325 mm	35 mm	300 mm × 16 mm ID
Dimensions, internal	90 × 390 × 47 mm (W × H × D)			
Safety	Self-check and auto-calibration at power-on, selectable turn-off temperature			
Leak sensor	Gas sensor, adjustable sensitivity, acoustic signal, turn-off switch			

\* total outer dimensions of the column including screw caps

## Fluorescence Detector (optional)

Thermostating		
Light Source	Xenon lamp	
Wavelength Range	200 to 650 nm	
Spectral Bandwidth	20 nm	
Wavelength Accuracy	±2 nm	
Wavelength Reproducibility	±0.2 nm	
S/N	Water Raman peak S/N of 1200 or greater	
Cell (Capacity, Pressure resistance, wet- ted material)	12 mL; 2 MPa {20 kgf/cm <sup>2</sup> }; SUS316L, PTFE (fluorocarbon polymers), quartz	
Simultaneous Monitoring of 2 Wavelengths	Measured Wavelengths	Any two wavelengths within the range of 200 to 650 nm
	Sampling Frequency	0.5 sec for one wavelength
Operating Temperature Range	4–35 °C	
Dimensions	W 26 cm × H 21 cm × D 42 cm	
Weight	16 kg	

## CIMac™ pDNA Analytical Column

Attribute	
Housing Material	Precision-engineered stainless steel
Connector	10-32 UNF coned port, 1/16" OD connection, straightforward connection to any HPLC
Flow Design	Symmetrical, operation in both directions possible, axial flow
Operation	With HPLC   FPLC systems
Volume	0.3 mL
Channel Width	1.4 or 6 µm
Base Matrix	Polymethacrylate monolith
Chemistry	Weak AEX

# Ordering Information

Item	Description	Package   Volume   Quantity   Size	Order Number
PATfix® pDNA Platform	HPLC solution for pDNA analytics	Includes system with uv and conductivity   pH detectors, column oven, PATfix software, 5 CIMac pDNA columns, user guides, methods and pDNA standards. Note: fluorescence detector is ordered separately. PC is not included.	PAT0029
Computer	PC, Windows 10 Professional, Monitor		PAT0006
Installation, Quantification and 2 Days Training			PAT0002
Column Oven	5 – 85 °C, up to 8 columns		PAT0030
Fluorescence Detector	200 – 650 nm, interface box		PAT0023
CIMac™ pDNA Column	0.3 mL, 1.4 µm channels		150.8501-1.4
CIMac™ pDNA Column	0.3 mL, 2 µm channels		150.8501-2
CIMac™ pDNA Column	0.3 mL, 6 µm channels		150.8501-6

## Related Products

### CIMmultus® Process Packs

	HiP <sup>2</sup> Plasmid Process Pack™
Column volume	1 – 8,000 mL
Included chemistries	CIMmultus® DEAE CIMmultus® C4-HLD
Additional components	Detailed process instructions

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 **For More Information, Visit**  
[www.sartorius.com](http://www.sartorius.com)