



ADVANCED BIOSCIENCES

Enhancing biopharmaceutical production and purification

AMBERCHROM™ *Profile*™ HPLC Columns

INSTRUCTIONS FOR USE

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INTRODUCTION

AMBERCHROM™ *Profile*™ HPLC columns are rugged, high performance products designed for separation of biomolecules.

The information contained in this manual are intended to guide you in the care and use of your column.

With proper care, as described in this manual, maximum column life can be expected. Rohm and Haas columns are guaranteed to be free from defects for 90 days from receipt of the column.

RECEIPT OF THE COLUMN

Upon receipt of the column, please verify the following:

- The column you received is the column you ordered.
- There is no physical damage to the column which may have occurred during shipping.
- That the column performs according to the specification sheet.

Each AMBERCHROM *Profile* HPLC column is individually packed and tested to ensure the highest column quality. Every column is supplied with its Test Chromatogram and a Specification Sheet which indicates column serial number and identity, testing conditions, and operating parameters.

The warranty period begins upon receipt of the column. Testing is especially important if the column is to be placed in storage. Test the column using the same conditions in the test chromatogram. Chromatographic performance depends on the entire system, not just the column.

Columns are QC tested using optimum conditions to minimize band spreading from “extra column effects”. Variations from the Rohm and Haas test data may be due to extra-column effects created by the design of the chromatography system. If you have any questions regarding your test results or the column quality, or if there are signs of damage, please contact your local distributor or Rohm and Haas immediately.

COLUMN INSTALLATION

Please follow the following instructions when installing a new AMBERCHROM *Profile* HPLC column:

- Flush the HPLC pump and line thoroughly with filtered and degassed mobile phase. Make sure there are no air bubbles in the system.
- Connect the column to the injector with the column in the proper flow direction (please note the flow arrow on the column). Leave the outlet of the column unattached.
- Slowly ramp the flow rate of the pump to a normal operating flow rate over the course of five minutes.
- When there is flow from the column outlet, attach the column to the detector.
- Equilibrate the column by passing 5-10 column volumes of mobile phase at a normal flow rate.

COLUMN EVALUATION

In order to verify performance of your new AMBERCHROM *Profile* HPLC column the following recommendations are provide below.

Recommended Efficiency Probes and Mobile Phases

Efficiency Probe	Mobile Phase
1% sodium chloride solution	20% ethanol/80% Milli-Q® water
5% acetone solution	60% acetonitrile/40% Milli-Q® water
1mg/mL methyl paraben solution	45% acetonitrile/55% Milli-Q® water

* 4.6mm I.D. use a 10µL injection volume, 10mm I.D. use a 50µL injection volume, 20mm I.D. use a 200µL injection volume

Efficiency and Asymmetry Targets

Packing	Efficiency	Asymmetry
10µm	>30,000 plates/M	0.8 – 1.6
20µm	>18,000 plates/M	0.8 – 1.6
30µm	>10,000 plates/M	0.8 – 1.6

COLUMN OPERATION

Flow rate range	4.6mm I.D. x 25cm L	0.1 – 0.5mL/min
	10mm I.D. x 25cm L	0.5 – 2.5mL/min
	20mm I.D. x 25cm L	2.0 – 10mL/min
Flow rate maximum	4.6mm I.D. x 25cm L	1.5mL/min
	10mm I.D. x 25cm L	5.0mL/min
	20mm I.D. x 25cm L	15mL/min
Pressure maximum	900 psi (60 bar)	
pH range	1 – 14	
Solvent range	5 – 100 %	
Solvent Compatibility	ethanol, ethanol, acetonitrile, isopropanol, n-propanol	
Temperature Range	4 – 60°C	
AVOID	Any strong oxidizing reagents, such as nitric acid. For stainless steel columns, avoid use of high concentrations of acids or halogenated salts	

COLUMN CLEANING

For routine cleaning, AMBERCHROM *Profile* HPLC columns can be cleaned using 3 – 5 column volumes of 95% acetonitrile at an intermediate flow rate. For more extensive cleaning, AMBERCHROM *Profile* HPLC columns can be cleaned with 3 – 5 column volumes of 0.5M NaOH/10% acetonitrile at a low flow rate, and then rinsed with 95% acetonitrile.

COLUMN STORAGE

AMBERCHROM *Profile* HPLC columns should be stored in an appropriate solvent and with the end-fittings tightly plugged. Please store them in a cool, dry area that is free from vibrations. Any salts or ion pairing agents, such as TEAA, should be flushed from the column with 5 to 10 column volumes of mobile phase.

SCALING UP A SEPARATION

To scale up a separation, it is important to scale according to the column internal diameter. The flow rate and column loading can then be adjusted so that the separation will be consistent at different scales. The chart can be used to determine the proper flow rate and loading.


Internal diameter	Scaling factor
4.6 mm	1x
10 mm	5x
20 mm	20x

Example Calculation

To scale up a separation from a 4.6mm ID x 25cm L column with a 0.5mL/min flow rate and a 1mg load to a 10mm ID x 25cm L column, the calculation would be as follows:

- Flow Rate: $0.5\text{mL}/\text{min} \times 5 = 2.5\text{mL}/\text{min}$
- Loading: $1\text{mg} \times 5 = 5\text{mg}$

ORDERING INFORMATION



Part Number	Description	Particle size	Dimensions
10237932	Amberchrom <i>Profile</i> HPR1046250	10µm	4.6mm x 25cm
10237938	Amberchrom <i>Profile</i> HPR1010250	10µm	10mm x 25cm
10237940	Amberchrom <i>Profile</i> HPR1020250	10µm	20mm x 25cm
10225950	Amberchrom <i>Profile</i> XT2046250	20µm	4.6mm x 25cm
1023774	Amberchrom <i>Profile</i> XT2010250	20µm	10mm x 25cm
1023775	Amberchrom <i>Profile</i> XT2020250	20µm	20mm x 25cm
10248207	Amberchrom <i>Profile</i> XT3046250	30µm	4.6mm x 25cm
10237909	Amberchrom <i>Profile</i> XT3010250	30µm	10mm x 25cm
10237941	Amberchrom <i>Profile</i> XT3020250	30µm	20mm x 25cm

ROHM AND HAAS WARRANTY

Rohm and Haas products are warranted to meet the stated specifications. They are not warranted, nor does Rohm and Haas assume liability, for misuse. No other warranty or representation is implied or expressed by Rohm and Haas for its products with respect to merchantability, fitness for a particular purpose or any other matter. Rohm and Haas shall not under any circumstances be liable for any incidental, consequential, or compensatory damage arising from the use of, or in conjunction with, its products. The maximum liability which can be assumed by Rohm and Haas for breach of warranty shall be the invoice price of the product.

Rohm and Haas warrants its quality columns in accordance with the following terms and conditions. Rohm and Haas will replace or refund charges on any column (at our discretion), at no cost if a column fails to perform satisfactorily. Columns being returned must have prior return authorization granted by Rohm and Haas.

Defective products must be accompanied by a written explanation of failure. Approval is subject to the following exclusions:

- All columns must be tested upon receipt and all deficiencies must be reported to Rohm and Haas no later than 30 days after date of receipt of the column.
- Maximum warranty period is limited to 90 days on HPLC columns unless previously agreed upon.
- Removal of column end-fittings automatically voids column warranty.
- Column performance warranty is limited to the conditions of the original test chromatogram.
- Physical damage to the column due to misuse, abuse, or mishap, including mechanical shock.
- Chemical damage to the packing material due to operation or incorrect chemical conditions, temperatures, or pressures.
- Failure due to high backpressures caused by improper solvent or sample filtration practices causing particulate build-up or precipitation in the column or end-fitting.
- Incorrect selection of packing material made by customer for their particular use or incompatibility of equipment, etc.
- For products supplied by but not manufactured by Rohm and Haas, the warranty is limited by the terms of the original manufacturer's warranty.

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