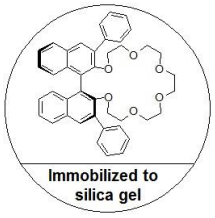
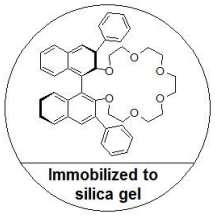
INSTRUCTION MANUAL FOR CROWNPAK® CR-I(+) and CROWNPAK® CR-I(-)

<Reversed Phase>

Please read this instruction sheet completely before using these columns

These columns can also be used in normal phase mode.
Please refer to the corresponding instruction sheet for details.

Column Description

CROWNPAK® CR-I(+)	CROWNPAK® CR-I(-)
	

Chiral Crown Ether **immobilized** to **5 µm silica-gel**

Shipping solvent: H_2O/MeOH 95:5 (v/v)

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, are included on a separate (enclosed) page.

Chiral Recognition

Chiral recognition can be achieved with CROWNPAK® CR-I(+) / CR-I(-) columns when a complex is formed between the crown ether and an ammonium ion ($-NH_3^+$) derived from a sample, under acidic conditions.
These columns can resolve not only amino acids but also compounds bearing a primary amino group near the chiral center.

With CROWNPAK® CR-I(+), the D-form of amino acids always elutes in first position.
Using CROWNPAK® CR-I(-) will result in an inversion of the elution order.

Operating Restrictions

	3.0 x 150 mm Analytical column
Flow rate direction	As indicated on the column label
Typical flow rate	0.2 ~ 0.4 mL / min
Pressure limitation❶	Should be maintained < 300 Bar (4350 psi) for maximum column life
Temperature	-5 to 40°C

❶The back pressure value that should be taken into account is the one generated by the column itself.
This value is measured by calculating the difference between the pressure of [LC system + column] and the pressure of the LC system free of the column.

Operating Procedure / Reversed Phase

A. Mobile phase

CROWNPAK CR-I(+) and CR-I(-) columns allow *free choice* of any miscible solvents, which are commonly used for HPLC analyses, to compose the mobile phase. When developing methods, we would recommend reversed phase mode as a first choice. Normal phase mode is a second choice.

Aqueous solution❶	$HClO_4$ aqueous solution
Organic solvent❷	CH_3CN, MeOH, EtOH, IPA, THF
Typical starting conditions❸	Aqueous solution of $HClO_4$ (pH=1.5) / CH_3CN = 80 / 20 (v/v)

CH_3CN: Acetonitrile, MeOH: Methanol, EtOH: Ethanol, IPA: 2-Propanol, THF: Tetrahydrofuran

❶ ⇨ Typical pH range of the mobile phase: from pH 1 to pH 2 (column stable up to pH 7).
⇨ Lower pH will result in longer retention times but a shorter column life. Choose the highest pH giving a satisfactory separation to prolong column life time.
⇨ Decreasing the temperature is also effective to increase the selectivity.
⇨ Other acids such as nitric acid and TFA can also be used. However, we recommend to use perchloric acid preferably which gives, in most cases, better resolutions and also for its low UV-absorption.

❷ ⇨ The elution power of organic modifiers for these columns is in the descending order of
THF > CH_3CN > IPA > EtOH > MeOH.
⇨The use of alcohols causes the back pressure to be significantly higher compared to CH_3CN due to their high viscosity in mixtures with water.

❸ ⇨ The aqueous solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.
⇨Retention can be adjusted by changing the proportion of organic solvent.

B. Examples of mobile phase preparation:

Hereafter are some indications to prepare the aqueous solution. The resulting pH value of the solution must be measured and adjusted for accuracy.

a) pH = 1.0

Weigh out 16.3 grams of commercially available perchloric acid (70%) and diluted to 1 L with distilled water.

b) pH = 2.0

100 mL of pH 1.0 solution is diluted to 1 L with distilled water.

c) pH = 1.5

316 mL of pH 1.0 solution is diluted to 1 L distilled water.

d) pH = 1.3

500 mL of pH 1.0 solution is diluted to 1 L distilled water.

Notes: *- Mobile phases should be completely degassed or thoroughly purged with helium.*

- Capacity factors on these columns depend on the hydrophobic nature of samples. Hydrophobic compounds are more retained compared to the hydrophilic ones. When your sample shows a little retention and a poor resolution the separation may be improved by decreasing the pH of the mobile phase (and column temperature).

- Although this column is not damaged by K^+ ions, the chiral recognition may be disturbed when present. The use of mobile phases containing K^+ ions should be avoided.

Column Care / Maintenance

- The use of a guard filter is highly recommended for maximum column life. The guard filter is in common use between CROWNPAK® CR-I(+) and CR-I(-).
- Samples should preferably be dissolved in the mobile phase. The mobile phase and the sample solution should be filtered through a membrane filter of approximately 0.5 µm porosity to ensure that there is no precipitate before using.
- Injecting a too concentrated sample solution will lead to a low column efficiency.
- When washing is required, use the solvent which can dissolve the sample such as pure methanol or acetonitrile at 0.2 mL/min for about 2 hours (room temperature).
- The column and the guard filter should be immediately flushed with a mobile phase with no acidic additive after use.
- H_2O / MeOH = 95 / 5 (v/v) can be used as a storage solvent when used continuously under reversed phase.

Operating this column in accordance with the guidelines outlined here will result in a long column life.

⇨ If you have any questions about the use of this column, or encounter a problem, contact:

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