

## INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ

### <Reversed-Phase>

**Please read this instruction manual completely before using these columns.**  
These columns can also be used in normal phase and SFC. Please refer to the corresponding instruction manual for details.

#### Column Description

AMYLOSE-BASED		CELLULOSE-BASED
Immobilized on 5 μm silica gel		Immobilized on 5 μm silica gel
<b>CHIRALPAK® IA</b>	<b>CHIRALPAK® ID</b>	<b>CHIRALPAK® IB</b> <b>CHIRALPAK® IB N-5</b>
Amylose tris(3,5-dimethylphenylcarbamate)	Amylose tris(3-chlorophenylcarbamate)	Cellulose tris(3,5-dimethylphenylcarbamate)
<b>CHIRALPAK® IE</b>	<b>CHIRALPAK® IF</b>	<b>CHIRALPAK® IC</b>
Amylose tris(3,5-dichlorophenylcarbamate)	Amylose tris(3-chloro-4-methylphenylcarbamate)	Cellulose tris(3,5-dichlorophenylcarbamate)
<b>CHIRALPAK® IG</b>	<b>CHIRALPAK® IH</b>	<b>CHIRALPAK® IJ</b>
Amylose tris(3-chloro-5-methylphenylcarbamate)	Amylose tris[(S)-α-methylbenzylcarbamate]	Cellulose tris(4-methylbenzoate)

Shipping Solvent:

1. Hexane/IPA = 90:10 (v/v) for analytical columns (2.1 mm i.d x 150 mm, 4.6 mm i.d. x 150 and 250 mm), guard, and semi-prep columns
2. 100% Methanol for analytical (4.6 mm i.d. x 50 and 100 mm) columns

All columns have been pre-tested before packaging. Test parameters and results, as well as the Column Lot Number, were included with the column when purchased.

\*Because different columns, including custom columns, can be shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol, at the typical flow rate listed below, before their first use to avoid any damage.\*

**THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS**

## Operating Instructions

	150 x 2.1 mm i.d. Analytical Column	50 x 4.6 mm i.d. 100 x 4.6 mm i.d. 150 x 4.6 mm i.d. 250 x 4.6 mm i.d. Analytical Columns	250 x 10 mm i.d.① 250 x 20 mm i.d.① 250 x 30 mm i.d.① 250 x 50 mm i.d.① Semi-Prep Columns
<b>Guard</b>	//	10 x 4.0 mm i.d. Guard Cartridge	20 x 10 mm i.d. 50 x 21 mm i.d. 50 x 30 mm i.d. Guard Column
Flow Rate Direction	As indicated on the column label		
Typical Flow Rate	0.1-0.5 ml/min	0.5-2.5 ml/min	5 ml/min (10 mm i.d.) 20 ml/min (20 mm i.d.) 42 ml/min (30 mm i.d.) 118 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life <a href="#">Adapt flow rates to column size.</a>		
Temperature	0 to 40°C		
Column Fitting	Please contact <a href="#">Technical Support</a> for details		

① When using a semi-preparative column, it is highly recommended to discard at least the first 150 ml (for 250 x 10 mm i.d) or 500 ml (for 250 x 20 mm i.d) of eluent at the beginning of each preparative work.

② The column pressure is the total pressure minus the system pressure. At a given temperature, the column back pressure is linearly proportional to the flow rate.

## Switching Between RP and NP or SFC

To switch from reversed-phase to normal phase or SFC, and vice versa, the column should be carefully flushed with miscible solvent, such as ethanol or isopropanol. The column should be flushed in a similar manner with ethanol or isopropanol when initially received after purchase, before first used in reversed-phase, as it could contain a hexane/alcohol mixture.

It is highly recommended that the **regeneration procedure** described in the instruction manual for normal phase (located in the column care and maintenance section) be used when switching from reversed-phase to normal phase or SFC. Before applying this procedure, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.

**A - Mobile Phases / For Both UV and Mass Detections**

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ④
<b>CHIRALPAK® IA</b> <b>CHIRALPAK® ID</b> <b>CHIRALPAK® IE</b> <b>CHIRALPAK® IF</b> <b>CHIRALPAK® IG</b> <b>CHIRALPAK® IH</b>  <b>CHIRALPAK® IB</b> <b>CHIRALPAK® IB N-5</b> <b>CHIRALPAK® IC</b> <b>CHIRALPAK® IJ</b>	Aqueous Solution ①	HCOOH aq. pH 2.0	Water	20 mM NH <sub>4</sub> HCO <sub>3</sub> aq. pH 9.0 adjusted with a basic additive ①
	Organic Modifier ②	ACN or MeOH or EtOH or IPA or THF		
	Typical Starting Conditions ③	Aqueous solutions 60% ACN 40% ⑤		

☞ NOTE 1: If you cannot achieve sufficient resolution, try the complementary aqueous solutions

**B – Complementary Aqueous and Buffer Solutions / For UV Detection Only**

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ④
<b>CHIRALPAK® IA</b> <b>CHIRALPAK® ID</b> <b>CHIRALPAK® IE</b> <b>CHIRALPAK® IF</b> <b>CHIRALPAK® IG</b> <b>CHIRALPAK® IH</b>  <b>CHIRALPAK® IB</b> <b>CHIRALPAK® IB N-5</b> <b>CHIRALPAK® IC</b> <b>CHIRALPAK® IJ</b>	Aqueous Solution ①	50 mM Phosphate Buffer pH 2.0 OR H <sub>3</sub> PO <sub>4</sub> aq. pH 2.0 OR 100 mM KPF <sub>6</sub> (or NaPF <sub>6</sub> ) aq. pH 2.0 adjusted with H <sub>3</sub> PO <sub>4</sub>	Water	20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ⑥ OR 100 mM KPF <sub>6</sub> (or NaPF <sub>6</sub> ) aq.

☞ NOTE 2: The concentration of all the buffering salt should be less than 500 mM.

- ① Refer to **section C** for the preparation of an aqueous solution and choice of basic additives.
- ②
  - ☐ It is recommended to use ACN to start the investigation
  - ☐ The elution power of organic modifiers for these columns is in the descending order of ACN > EtOH > MeOH: 50%ACN ≈ 65-70%EtOH ≈ 75-80%MeOH.
  - ☐ The use of other organic solvents, **except THF**, has not been investigated and could be harmful to the columns.
  - ☐ The use of alcohols causes the back pressure to be significantly higher compared to ACN due to their higher viscosity in mixtures with water.
- ③
  - ☐ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present in the mobile phase.
  - ☐ Lowering the column temperature may increase the retention time and the selectivity.
  - ☐ Increasing the column temperature and decreasing the flow rate may increase the resolution.
- ④
  - ☐ To maximize column life, the use of a guard cartridge or guard column is essential when basic conditions are employed.
  - ☐ The use of strong basic conditions (> pH 9) must be avoided, as they are known to damage the silica gel matrix.
  - ☐ When these columns are used at pH > 7, **the temperature should be maintained between 5°C and 25°C for maximum column life.**

- High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** from the solution, and lead to subsequent clogging of the column (refer to the table below).

Water / Organic Modifier	Buffer solution / Organic Modifier
90 / 10 to 0 / 100	90 / 10 to 15 / 85

- Do not use a phosphate buffer for pH > 8. When pH 9 is necessary, the use of the ammonium bicarbonate solution or borate buffer is recommended for maximum column life.

## C – Buffer Preparation – Examples

### ➤ Preparation of pH 2 Phosphate buffer:

**Solution A:** 50 mM potassium dihydrogenphosphate

3.40g  $\text{KH}_2\text{PO}_4$  / FW 136.09, make up the volume to 500ml with HPLC grade water

**Solution B:** phosphoric acid ( $\text{H}_3\text{PO}_4$  85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

### ➤ Preparation of pH 2 $\text{KPF}_6$ ( $\text{NaPF}_6$ ) solution:

**Solution A:** 100 mM potassium (sodium) hexafluorophosphate

9.20g  $\text{KPF}_6$  / FW 184.06 or 8.40g  $\text{NaPF}_6$  / FW 167.95, make up the volume to 500 ml with HPLC grade water

**Solution B:** phosphoric acid ( $\text{H}_3\text{PO}_4$  85% by weight)

Adjust the pH of solution A to a value of 2.0 using solution B.

### ➤ Preparation of pH 9 Ammonium bicarbonate solution:

**Solution A:** 20 mM ammonium bicarbonate

0.78g  $\text{NH}_4\text{HCO}_3$  / FW 78.05, make up the volume to 500 ml with HPLC grade water

**Solution B** Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia ( $\text{NH}_3$ ) and so on.

*\* DEA tends to give better peak shape than other bases.*

Adjust the pH of solution A to a value of 9.0 using solution B.

### ➤ Preparation of pH 8 Phosphate buffer:

**Solution A:** 20 mM potassium hydrogenophosphate

1.74g of  $\text{K}_2\text{HPO}_4$  / FW 174.18, make up the volume to 500 ml with HPLC grade water

**Solution B:** 20 mM potassium dihydrogenophosphate

1.36g  $\text{KH}_2\text{PO}_4$  / FW 136.09, make up the volume to 500 ml with HPLC grade water.

Adjust the pH of solution A to a value of 8.0 using solution B.

### ➤ Preparation of pH 9 Borate buffer:

**Solution A:** 20 mM sodium tetraborate decahydrate

3.81g of  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$  / FW 381.37, make up the volume to 500 ml with HPLC grade water

**Solution B:** 20 mM boric acid

0.62g  $\text{H}_3\text{BO}_3$  / FW 61.83, make up the volume to 500 ml with HPLC grade water

Adjust the pH of solution A to a value of 9.0 using solution B.

## Column Care / Maintenance

- ❑ The use of a guard cartridge or guard column is highly recommended for maximum column life.
- ❑ 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 0.5µm porosity to ensure that there is no precipitate before using.

Following extensive use of the column in multiple solvents, there may be a change in separation reproducibility. In order to ensure consistent performance, a regeneration method may be implemented to eliminate any change in chiral recognition due to the history of the column (mobile phases, additives...).

**For detailed Regeneration Procedures, please [click here](#)**

## Column Storage

- ❑ For column storage and/or switching to 100% organic solvent, any traces of salts should be removed by flushing the column with a mobile phase which doesn't contain any salts or buffers, for instance Water/ACN = 60/40 (v/v).
- ❑ Columns can be stored with ends capped in the additive-free mobile phase, or the shipping solvent, at room temperature.

*Operating these columns in accordance with the guidelines outlined here will result in a long column life.*

⇒ If you have any questions about the use of these columns, or encounter a problem, contact:

In the USA: [questions@cti.daicel.com](mailto:questions@cti.daicel.com) or call 800-6-CHIRAL

In the EU: [cte@cte.daicel.com](mailto:cte@cte.daicel.com) or call +33 (0) 3 88 79 52 00

In India: [chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com) or call +91 84 1866 0700 & 703

### Locations:

#### **North/Latin America**

Chiral Technologies. Inc.  
1475 Dunwoody Dr. Ste 310  
West Chester, PA 19380  
800 6 CHIRAL  
Tel: 610-594-2100  
Fax: 610-594-2325  
[chiral@cti.daicel.com](mailto:chiral@cti.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

#### **Europe**

Chiral Technologies Europe SAS  
Parc d'Innovation  
160, Bd Gonthier d'Andernach CS 80140  
67404 Illkirch Cedex France  
Tel: +33 (0) 3 88 79 52 00  
Fax: +33 (0) 3 88 66 71 66  
[cte@cte.daicel.com](mailto:cte@cte.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

#### **India**

Daicel Chiral Technologies (India) Pvt Ltd  
Survey No. 542/2 IKP Knowledge Park, Turkapally,  
Shamirpet Mandal, Medchal-Malkajgiri District,  
Hyderabad-500101. Telangana, India  
Tel: +91 84 1866 0700 & 703  
Fax: +91 84 1866 0730  
[chiral@chiral.daicel.com](mailto:chiral@chiral.daicel.com)  
[www.chiraltech.com](http://www.chiraltech.com)

**CHIRALCEL, CHIRALPAK, CROWNPAK and DAICEL DCpak** are registered trademarks of **DAICEL CORPORATION**