

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

<Supercritical Fluid Chromatography (SFC)>

Please read this instruction manual completely before using these columns.
These columns can also be used in reversed-phase and normal phase. 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
CHIRALPAK® IA	CHIRALPAK® ID	CHIRALPAK® IB CHIRALPAK® IB N-5
Amylose tris(3,5-dimethylphenylcarbamate)	Amylose tris(3-chlorophenylcarbamate)	Cellulose tris(3,5-dimethylphenylcarbamate)
CHIRALPAK® IE	CHIRALPAK® IF	CHIRALPAK® IC
Amylose tris(3,5-dichlorophenylcarbamate)	Amylose tris(3-chloro-4-methylphenylcarbamate)	Cellulose tris(3,5-dichlorophenylcarbamate)
CHIRALPAK® IG	CHIRALPAK® IH	CHIRALPAK® IJ
Amylose tris(3-chloro-5-methylphenylcarbamate)	Amylose tris[(S)-α-methylbenzylcarbamate]	Cellulose tris(4-methylbenzoate)
Shipping Solvent:		
<ol style="list-style-type: none"> 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 (see column transfer conditions between LC and SFC on page 5).

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
Guard	//	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 in SFC	0.5-1.0 ml/min	1.0-5.0 ml/min	15 ml/min (10 mm i.d.) 60 ml/min (20 mm i.d.) 120 ml/min (30 mm i.d.) 350 ml/min (50 mm i.d.)
Pressure Limitation②	Should be maintained < 300 Bar (4350 psi) for maximum column life Adapt flow rates to column size.		
Temperature	0 to 40°C		
Column Fitting	Please contact Technical Support 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 relevant pressure value is the one generated by the column itself (pressure drop). The pressure drop is the difference between the inlet pressure (P_{inlet}) and the outlet pressure (P_{outlet}) in the system. The pressure drop generated by the system alone (without any column) has to be subtracted from the total value (system + column).

The column can be operated up to 300 Bar (pressure drop). However, it is necessary to check if the SFC system has been designed to withstand these conditions. The flow rate has to be adapted considering the pressure drop in the column (this pressure being dependent upon flow rate as well as the amount and type of modifier in the mobile phase).

Method Development / SFC

A - Mobile Phases

CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO₂)*, progressing from the traditional solvents used with other DAICEL columns (mixtures of CO₂ with alcohols or acetonitrile (CH₃CN)) to mobile phases containing CO₂ with methyl *tert*-butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform (CHCl₃), and ethyl acetate (EtOAc), among others.

B - Method Development - Screening

When developing methods, we would recommend a screening approach.

1. The conditions described in Table 1 should be used as a **Primary Screening**.
2. If the compound or compound series are not soluble in any of these mobile phases, we recommend trying the **Primary Screening** with the product dissolved in a stronger solvent (DCM/alcohol...).

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO ₂ / MeOH	CO ₂ / EtOH	CO ₂ / 2-PrOH	CO ₂ / ACN ^①
Typical Starting Conditions	80:20	80:20	80:20	70:30 ^①
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60 ^①

① Alcohols can be added into ACN to enhance the eluting strength for strongly retained compounds.

If a suitable chiral separation is not found using the Immobilized Primary Screening strategy, we recommend progressing to an Immobilized Secondary Screening using the following conditions:

Table 2. Immobilized Secondary Screening Solvents

Secondary Solvent Mixtures	CO ₂ / THF	CO ₂ / (DCM+MeOH 90:10)	CO ₂ / (EtOAc+MeOH 90:10)	CO ₂ / (MtBE+MeOH 80:20)
Typical Starting Conditions	75:25	80:20	80:20	75:25
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60

Notes: The alcohol content and type (MeOH, EtOH and 2-PrOH) can be used to modulate retention and recognition. THF can be added into DCM and EtOAc to enhance the eluting strength for strongly retained compounds.

All solvent proportions indicated in this manual are by volume.

C – General Comments

- ⇒ Only immobilized CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ are suitable for the Secondary Screening.
- ⇒ Additional modifiers such as CHCl₃, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, and IJ.
- ⇒ The typical starting conditions consist of mobile phases of upper middle eluting strength. Under such conditions, most of the analytes can be eluted within a reasonable time range with a good probability of full resolution of the enantiomers.
- ⇒ It is important to check your SFC system (seals...) is compatible with all types of solvents and to take into account UV cut-off of certain solvents, in order to avoid detection issues. Detection with a regular UV detector may become difficult depending on a combination of sample and mobile phase (e.g. EtOAc, high percentages of DCM).

D – Additives

For basic samples, it is necessary to incorporate an additive into the mobile phase in order to optimize the chiral separation.

Acidic samples **do not always** require the presence of an additive. In fact, the acidic properties of the carbon dioxide (CO₂) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ^{①②}	Acidic Samples require Acidic additives ^①
Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid Formic acid

⇒ **STRONGLY BASIC solvent additives or sample solutions MUST BE AVOIDED**, because they are likely to damage the silica gel used in this column

① In practice, 1% of the additive is incorporated with the modifier. The total amount of additive into the mobile phase will be dependent upon the percentage of modifier. For example, if the mobile phase is CO₂ / EtOH = 90:10, with EtOH containing 1% of additive, then the mobile phase composition will be CO₂ / EtOH / additive = 90:10:0.1).

② For preparative purposes, it is recommended to use DEA or TEA as additives, due to their easy removal from the products by standard evaporation and drying systems.

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 modifier.
- ❑ Sample solutions should be filtered through a membrane filter of approximately 0.5µm porosity to ensure that there is no precipitate before use.

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 transfer between modes:

From LC to SFC

- Flush with 100% 2-PrOH at 0.25 ml/min^(*) for 45 min
- Flush with 100% CO₂ or CO₂+modifier at 0.25 ml/min^(*) for 45 min

From SFC to LC

- Flush with 100% 2-PrOH at 0.25 ml/min^(*) for 45 min
- Flush with the mobile phase at 0.25 ml/min^(*) for 45 min

() This is the recommended flow rate for a 4.6 mm i.d. analytical columns. The flow rate of all other inner diameter columns should be adjusted proportional according to the cross-sectional area of the column.*

Column Storage

- ❑ For column storage, remove the acidic or basic additives by flushing the column with several column volumes of 100% 2-PrOH or 100% methanol, without additives.
- ❑ 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 or call 800-6-CHIRAL

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

In India: 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
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
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
www.chiraltech.com

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