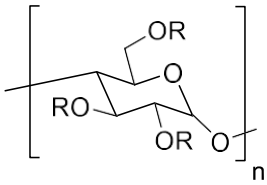
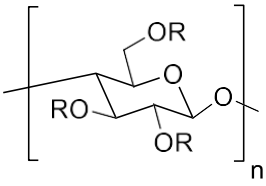
DAICEL
Chiral Technologies

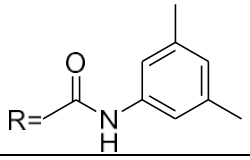
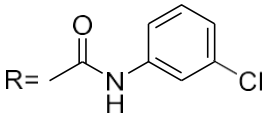
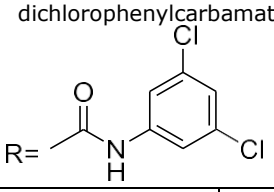
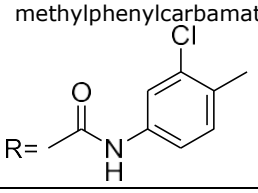
INSTRUCTION MANUAL FOR CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, IJ, IK, IM, and IN

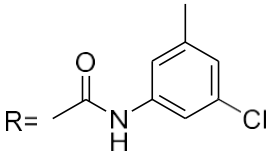
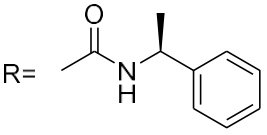
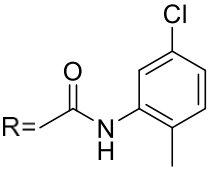
<Supercritical Fluid Chromatography (SFC)>

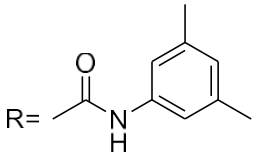
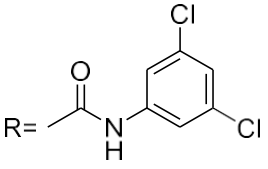
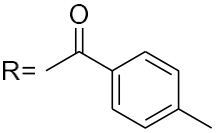
Please read this instruction manual completely before using these columns.

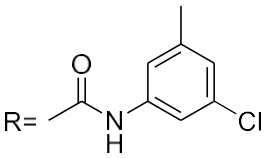
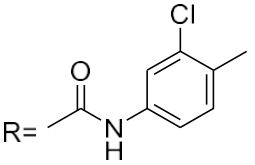
These columns can also be used in reversed-phase and normal phase. Please refer to the corresponding instruction manual for details.

AMYLOSE-BASED	**CELLULOSE-BASED**
	
Immobilized on 5 µm silica gel	**Immobilized on 5 µm silica gel**

CHIRALPAK® IA	**CHIRALPAK® ID**	**CHIRALPAK® IE**	**CHIRALPAK® IF**
Amylose tris (3,5-dimethyl-phenylcarbamate)	Amylose tris (3-chloro-phenylcarbamate)	Amylose tris(3,5-dichlorophenylcarbamate)	Amylose tris(3-chloro-4-methylphenylcarbamate)
			

CHIRALPAK® IG	**CHIRALPAK® IH**	**CHIRALPAK® IN**
Amylose tris(3-chloro-5-methyl-phenylcarbamate)	Amylose tris[(S)-α-methylbenzylcarbamate]	Amylose tris(5-chloro-2-methyl-phenylcarbamate)
		

CHIRALPAK® IB CHIRALPAK® IB N-5	**CHIRALPAK® IC**	**CHIRALPAK® IJ**
Cellulose tris(3,5-dimethylphenylcarbamate)	Cellulose tris(3,5-dichlorophenylcarbamate)	Cellulose tris(4-methylbenzoate)
		

CHIRALPAK® IK	**CHIRALPAK® IM**
Cellulose tris(3-chloro-5-methylphenylcarbamate)	Cellulose tris(3-chloro-4-methylphenylcarbamate)
	

Shipping Solvent:

1. **Hexane/Isopropanol (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 and prep columns (only applicable to HPLC columns).**
2. **100% Methanol for analytical (4.6 mm i.d. x 50 and 100 mm), guard, and semi-prep and prep columns (only applicable to SFC 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 4).

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 21 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	**50 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 (21 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 21 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, IJ, IK, IM, and IN can be used *with all ranges of organic miscible solvents as modifiers combined with supercritical carbon dioxide (CO_2),* progressing from the traditional solvents used with other DAICEL columns (mixtures of CO_2 with alcohols or acetonitrile (CH_3CN)) to mobile phases containing CO_2 with methyl *tert*- butyl ether (MtBE), tetrahydrofuran (THF), dichloromethane (DCM), chloroform ($CHCl_3$), 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 ❶
Typical Starting Conditions	80:20	80:20	80:20	70:30 ❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60 ❶

❶ 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, IJ, IK, IM, and IN are suitable for the Secondary Screening.

⇨ Additional modifiers such as $CHCl_3$, 1,4-Dioxane, Toluene, or Acetone can also be investigated with CHIRALPAK® IA, IB, IB N-5, IC, ID, IE, IF, IG, IH, IJ, IK, IM, and IN.

⇨ 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 **<u>do not always</u>** require the presence of an additive. In fact, the acidic properties of carbon dioxide (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶❷	Acidic Samples require Acidic additives❶
Diethylamine (DEA) Triethylamine (TEA)	Trifluoroacetic acid (TFA) Acetic acid (AcOH) Formic acid

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

❶ 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 CO_2/EtOH = 90-10, with EtOH containing 1% of additive, then the mobile phase composition will be CO_2 /EtOH/additive = 90-10-0.1.

❷ 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 0.5 µm 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…).

<u>For detailed Regeneration Procedures, please click here</u>

☞ ***<u>Column transfer between modes:</u>***

<u>From LC to SFC</u>

- Flush with 100% 2-PrOH at 0.25 ml/min(*) for 45min
- Flush with 100% CO_2 or CO_2+modifier at 0.25 ml/min(*) for 45min

<u>From SFC to LC</u>

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

(*) *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:

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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

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