DAICEL
Chiral Technologies

INSTRUCTION MANUAL FOR
CHIRALPAK® IA-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, IK-3

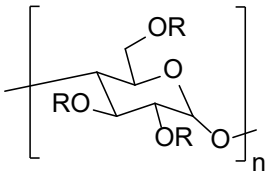
<Supercritical Fluid Chromatography (SFC)>

Please read this instruction sheet completely before using these columns.

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

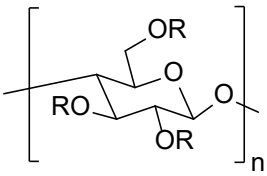
Column Description

AMYLOSE-BASED



Immobilized on **3 µm silica gel**

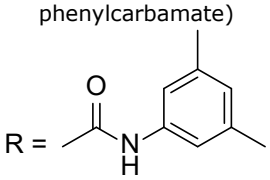
CELLULOSE-BASED



Immobilized on **3 µm silica gel**

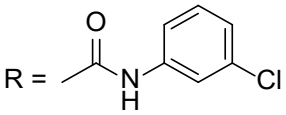
CHIRALPAK® IA-3

Amylose tris(3,5-dimethyl-phenylcarbamate)



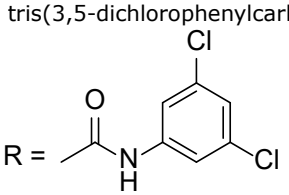
CHIRALPAK® ID-3

Amylose tris(3-chloro-phenylcarbamate)



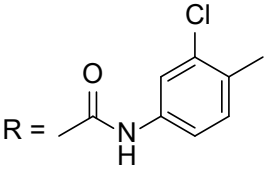
CHIRALPAK® IE-3

Amylose tris(3,5-dichlorophenylcarbamate)



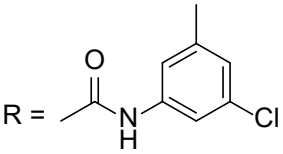
CHIRALPAK® IF-3

Amylose tris(3-chloro-4-methylphenylcarbamate)



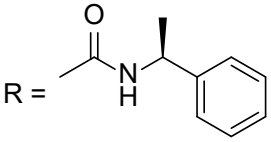
CHIRALPAK® IG-3

Amylose tris(3-chloro-5-methyl-phenylcarbamate)



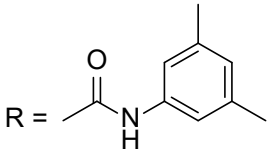
CHIRALPAK® IH-3

Amylose tris[(S)- α-methylbenzylcarbamate]



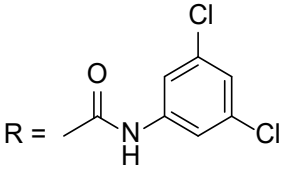
CHIRALPAK® IB-3
CHIRALPAK® IB N-3

Cellulose tris(3,5-dimethylphenylcarbamate)



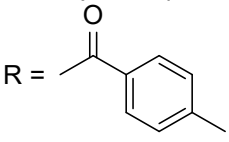
CHIRALPAK® IC-3

Cellulose tris(3,5-dichlorophenylcarbamate)



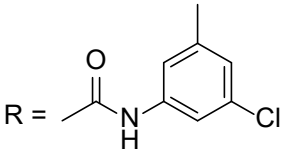
CHIRALPAK® IJ-3

Cellulose tris(4-methylbenzoate)



CHIRALPAK® IK-3

Cellulose tris(3-chloro-5-methyl-phenylcarbamate)



Shipping Solvent: **Hexane/IPA = 90:10 (v/v)**

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 are shipped in different solvents, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in SFC to avoid any damage (see column transfer conditions between LC and SFC on page 4).

THIS INSTRUCTION SHEET IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS.

Operating Instructions

	50 x 2.1 mm i.d. **100 x 2.1 mm i.d.** **150 x 2.1 mm i.d.** **250 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
Guard	**//**	**10 x 4.0 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
Pressure Limitation①	Please contact Technical Support for details	
Temperature	0 to 40°C	
Column Fitting	Please contact Technical Support for details	

① 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-3, IB-3, IB (N)-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, and IK-3 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 (ACN)) 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...).

3.

Table 1. Immobilized Primary Screening Solvents

Primary Solvent Mixtures	CO_2 / MeOH	CO_2 / EtOH	CO_2 /2-PrOH	CO_2 / ACN❶
Typical Starting Conditions	80:20	80:20	80:20	70:30❶
Advised Optimization Range	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60	99:1 to 40:60❶

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-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, and IK-3 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-3, IB-3, IB N-3, IC-3, ID-3, IE-3, IF-3, IG-3, IH-3, IJ-3, and IK-3 columns.
- ⇨ The typical starting conditions consist in 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 also important to ensure 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 (CO_2) are sometimes enough to elute the product properly.

Basic Samples require Basic additives ❶	Acidic Samples require Acidic additives ❶
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*

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).

Column Care / Maintenance

- ❑ The use of a 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 a detailed Regeneration Procedure, please click here.

☞ ***Column transfer between modes:***

From LC to SFC

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

From SFC to LC

- Flush with 100% EtOH 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

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