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

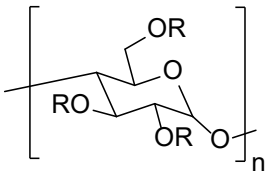
<Reversed-Phase>

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.

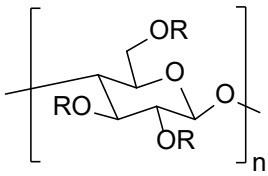
Column Description

AMYLOSE-BASED



Immobilized on **3 µm silica gel**

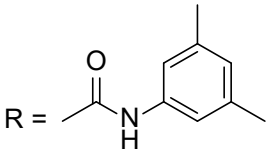
CELLULOSE-BASED



Immobilized on **3 µm silica gel**

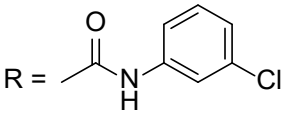
CHIRALPAK® IA-3

Amylose tris(3,5-dimethyl-phenylcarbamate)



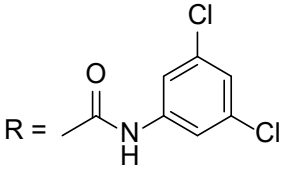
CHIRALPAK® ID-3

Amylose tris(3-chloro-phenylcarbamate)



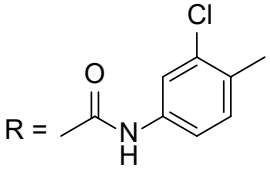
CHIRALPAK® IE-3

Amylose tris(3,5-dichlorophenylcarbamate)



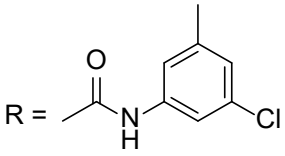
CHIRALPAK® IF-3

Amylose tris(3-chloro-4-methylphenylcarbamate)



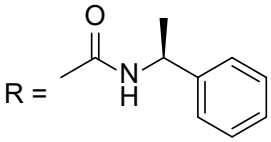
CHIRALPAK® IG-3

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



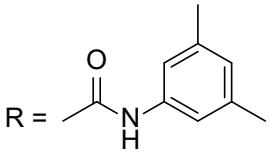
CHIRALPAK® IH-3

Amylose tris[(S)- α-methylbenzylcarbamate]



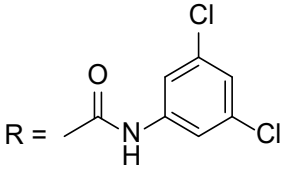
CHIRALPAK® IB-3
CHIRALPAK® IB N-3

Cellulose tris(3,5-dimethylphenylcarbamate)



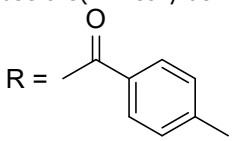
CHIRALPAK® IC-3

Cellulose tris(3,5-dichlorophenylcarbamate)



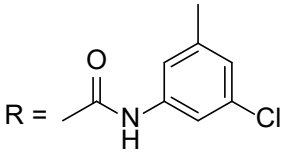
CHIRALPAK® IJ-3

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, 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

	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 Columns	**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 Cartridge
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 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** (link below in Column Care 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.

Method Development / Reversed-Phase

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	**NEUTRAL** Compounds	**BASIC** Compounds ❹
CHIRALPAK®IA-3 **CHIRALPAK®ID-3** **CHIRALPAK®IE-3** **CHIRALPAK®IF-3** **CHIRALPAK®IG-3** **CHIRALPAK®IH-3** **CHIRALPAK®IB-3** **CHIRALPAK®IB N-3** **CHIRALPAK®IC-3** **CHIRALPAK®IJ-3** **CHIRALPAK®IK-3**	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20 mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or IPA or THF		
	Typical Starting Conditions ❸	Aqueous solutions 60% ACN 40% ❺		

☞ *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 ❹
CHIRALPAK®IA-3 **CHIRALPAK®ID-3** **CHIRALPAK®IE-3** **CHIRALPAK®IF-3** **CHIRALPAK®IG-3** **CHIRALPAK®IH-3** **CHIRALPAK®IB-3** **CHIRALPAK®IB N-3** **CHIRALPAK®IC-3** **CHIRALPAK®IJ-3** **CHIRALPAK®IK-3**	Aqueous Solution ❶	50 mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100 mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20 mM Borate Buffer pH 9.0 OR 20 mM Phosphate Buffer pH 8.0 ❻ OR 100 mM KPF_6 (or $NaPF_6$) aq.

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

❶ Refer to **section C** for preparation of aqueous solution and choice of basic additives.

❷ ❑ 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 high viscosity in mixtures with water.

❸ ❑ Retention can be adjusted by changing the proportion of ACN. Retention may be very sensitive to the amount of ACN present into 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.

❹
- To maximize column life the use of a guard cartridge 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 25ºC for maximum column life.**

❺ High percentages of organic modifier in the mobile phase **<u>may precipitate the buffering salt</u>** from the solution, and lead to consequent 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**

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

C – Buffer Preparation – Examples

➢ *<u>Preparation of pH 2 Phosphate buffer</u>:*

Solution A: 50 mM potassium dihydrogenphosphate
3.40 g KH_2PO_4 / FW 136.09, make up the volume to 500 ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *<u>Preparation of pH 2 KPF_6 ($NaPF_6$) solution</u>:*

Solution A: 100m M potassium (sodium) hexafluorophosphate
9.20 g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500 ml with HPLC grade water
Solution B: phosphoric acid (H_3PO_4 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.

➢ *<u>Preparation of pH 9 Ammonium bicarbonate solution</u>:*

Solution A: 20 mM ammonium bicarbonate
0.78g NH_4HCO_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 (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.

➢ *<u>Preparation of pH 8 Phosphate buffer</u>:*

Solution A: 20 mM potassium hydrogenophosphate
1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500 ml with HPLC grade water
Solution B: 20 mM potassium dihydrogenophosphate
1.36g KH_2PO_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.

➢ *<u>Preparation of pH 9 Borate buffer</u>:*

Solution A: 20 mM sodium tetraborate decahydrate
3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500 ml with HPLC grade water
Solution B: 20 mM boric acid
0.62g H_3BO_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 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:

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