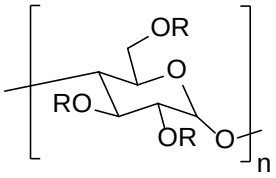
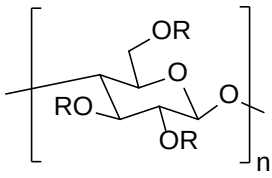
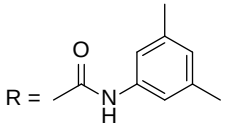
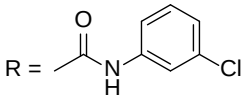
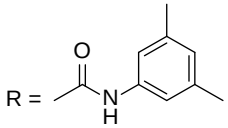
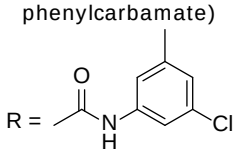
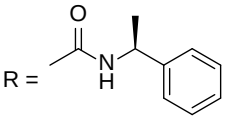
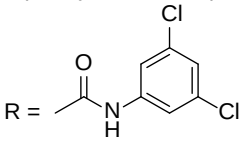
INSTRUCTION MANUAL FOR CHIRALPAK® IA-U, IB-U, IC-U, ID-U, IG-U, and IH-U

<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 **1.6 µm silica gel**		 **Immobilized** on **1.6 µm silica gel**
CHIRALPAK® IA-U	**CHIRALPAK® ID-U**	**CHIRALPAK® IB-U**
Amylose tris(3,5-dimethyl-phenylcarbamate) 	Amylose tris(3-chloro-phenylcarbamate) 	Cellulose tris(3,5-dimethyl phenylcarbamate) 
CHIRALPAK® IG-U	**CHIRALPAK® IH-U**	**CHIRALPAK® IC-U**
Amylose tris(3-chloro-5-methyl-phenylcarbamate) 	Amylose tris[(S)- α-methylbenzylcarbamate] 	Cellulose tris(3,5-dichloro 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 these columns are shipped in hexane/IPA, we recommend flushing them with 100% Ethanol or Isopropanol before their first use in reversed-phase to avoid any damage.

THIS INSTRUCTION MANUAL IS NOT APPLICABLE TO ANY OTHER DAICEL COLUMNS

Although these columns can be used with an HPLC system, it is highly recommended that a UHPLC system be utilized to preserve the best separation performance of the column.

General Recommendations

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

Although these columns can be used with an HPLC system, it is highly recommended that a UHPLC system be utilized to preserve the best separation performance of the column.

It is also highly recommended:
- to apply the **regeneration procedure** described in the Regeneration Procedure manual for switching back to normal phase or SFC. Before applying this protocol, any trace salts should be removed by flushing the column with a mobile phase that does not contain any salts / buffers, for example Water/ACN = 60/40, and then flushing with ethanol or isopropanol.
- to adjust the flow rate to ensure the column pressure < 700 bar.

Operating Instructions

	50 x 3.0 mm i.d. Analytical Columns	**100 x 3.0 mm i.d.** Analytical Columns
Flow Rate Direction	As indicated on the column label	
Typical Flow Rate	0.6 to 5.0 ml/min	0.6 to 2.6 ml/min
Temperature	0 to 40°C	
Column Pressure Limit①	700 bar (10150 psi)	
Column Fitting②	Upchurch or Parker-type	

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.

② **<u>It is highly recommended the matching fitting be used.</u>** Improperly matched fittings can create a void at the inlet, leading to significant extra-column band broadening. This effect is much more pronounced on these small, sub-2 µm particles, compared to larger particle sizes. Additionally, fitting mismatch can lead to significant leaking.

Method Development / Reversed-phase

A - Mobile Phases / For Both UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds ❹	**NEUTRAL** Compounds ❹	**BASIC** Compounds ❹
CHIRALPAK® IA-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® ID-U** **CHIRALPAK® IG-U** **CHIRALPAK® IH-U**	Aqueous Solution ❶	HCOOH aq. pH 2.0	Water	20mM NH_4HCO_3 aq. pH 9.0 adjusted with a basic additive❶
	Organic Modifier ❷	ACN or MeOH or EtOH or 2-PrOH 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-U **CHIRALPAK® IB-U** **CHIRALPAK® IC-U** **CHIRALPAK® ID-U** **CHIRALPAK® IG-U** **CHIRALPAK® IH-U**	Aqueous Solution ❶	50mM Phosphate Buffer pH 2.0 OR H_3PO_4 aq. pH 2.0 OR 100mM KPF_6 (or $NaPF_6$) aq. pH 2.0 adjusted with H_3PO_4	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❻ OR 100mM KPF_6 (or $NaPF_6$) aq.

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

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 the column life, it is essential to inject filtered clean sample solutions.** It is recommended to use at least a filter with a porosity of 0.5 µm.
- ❑ 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.**

❺ High percentages of organic modifier in the mobile phase **may precipitate the buffering salt** 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

➢ *Preparation of pH 2 Phosphate buffer:*

Solution A: 50mM potassium dihydrogenphosphate
3.40g KH_2PO_4 / FW 136.09, make up the volume to 500ml 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.

➢ *Preparation of pH 2 KPF_6 ($NaPF_6$) solution:*

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF_6 / FW 184.06 or 8.40g $NaPF_6$ / FW 167.95, make up the volume to 500ml 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.

➢ *Preparation of pH 9 Ammonium bicarbonate solution:*

Solution A: 20mM ammonium bicarbonate
0.78g NH_4HCO_3 / FW 78.05, make up the volume to 500ml 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.

➢ *Preparation of pH 8 Phosphate buffer:*

Solution A: 20mM potassium hydrogenophosphate

1.74g of K_2HPO_4 / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate

1.36g KH_2PO_4 / FW 136.09, make up the volume to 500ml 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: 20mM sodium tetraborate decahydrate

3.81g of $Na_2B_4O_7.10H_2O$ / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid

0.62g H_3BO_3 / FW 61.83, make up the volume to 500ml with HPLC grade water

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

Column Care / Maintenance

- 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…). This procedure should also be used when switching from reversed-phase to normal phase or SFC.

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

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