

INSTRUCTION MANUAL FOR CHIRALPAK® IA, CHIRALPAK® IB, CHIRALPAK® IC, CHIRALPAK® ID, CHIRALPAK® IE and CHIRALPAK® IF

<Reverse 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.**

Switching Between RP and NP Mode

To switch from reversed phase mode to normal phase mode, and vice versa, column should be carefully flushed with miscible solvent.

It is highly recommended to apply the **regeneration procedure** described in the instruction sheet for normal phase mode. Before applying this protocol, any traces of salts should be removed by flushing with a mobile phase that does not contain any salts / buffers.

Method Development / Reversed Phase

A - Mobile Phases / For UV and Mass Detections

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❶
CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IB CHIRALPAK® IC	Aqueous solution ❶	HCOOH aq. pH 2.0	Water	20mM NH ₄ HCO ₃ aq. pH 9.0 adjusted with a basic additive ❶
	Organic modifier ❷	CH ₃ CN or MeOH or EtOH or IPA or THF		
	Typical starting conditions ❸	Aqueous solutions CH ₃ CN	60%	40% ❹

☞ **NOTE 1:** If you cannot achieve sufficient resolution, try the complementary aqueous solutions

B – Complementary Aqueous and Buffer Solutions / For UV Detection

		ACIDIC (AMPHOTERIC) Compounds	NEUTRAL Compounds	BASIC Compounds ❶
CHIRALPAK® IA CHIRALPAK® ID CHIRALPAK® IE CHIRALPAK® IF CHIRALPAK® IB CHIRALPAK® IC	Aqueous solution ❶	50mM Phosphate Buffer pH 2.0 OR H ₃ PO ₄ aq. pH 2.0 OR 100mM KPF ₆ (or NaPF ₆) aq. pH 2.0 adjusted with H ₃ PO ₄	Water	20mM Borate Buffer pH 9.0 OR 20mM Phosphate Buffer pH 8.0 ❷ 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 CH₃CN to start the investigation
 - The elution power of organic modifiers for these columns is in the descending order of CH₃CN > EtOH > MeOH: 50%CH₃CN ≈ 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 CH₃CN due to their high viscosity in mixtures with water.
- ❸ □ Retention can be adjusted by changing the proportion of CH₃CN. Retention may be very sensitive to the amount of CH₃CN 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 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 KH₂PO₄ / FW 136.09, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H₃PO₄ 85% by weight)
Adjust the pH of solution A to a value of 2.0 using solution B.
- Preparation of pH 2 KPF₆ (NaPF₆) solution:

Solution A: 100mM potassium (sodium) hexafluorophosphate
9.20g KPF₆ / FW 184.06 or 8.40g NaPF₆ / FW 167.95, make up the volume to 500ml with HPLC grade water

Solution B: phosphoric acid (H₃PO₄ 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 NH₄HCO₃ / FW 78.05, make up the volume to 500ml with HPLC grade water

Solution B: Basic additive such as diethylamine (DEA), triethylamine (TEA), ammonia (NH₃) 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 K₂HPO₄ / FW 174.18, make up the volume to 500ml with HPLC grade water

Solution B: 20mM potassium dihydrogenophosphate
1.36g KH₂PO₄ / 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 Na₂B₄O₇·10H₂O / FW 381.37, make up the volume to 500ml with HPLC grade water

Solution B: 20mM boric acid
0.62g H₃BO₃ / 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

- Any traces of salts should be removed before column storage and /or before switching to 100% organic solvent (use Water/CH₃CN 60:40 (v/v) for instance)

Refer to main instruction for normal phase and column care/maintenance.