Operation Manual
Shodex IEC SP-FT 4A

(Please read this operation manual carefully to achieve the best and consistent column performance for a long time.)

Important Handling Instructions

Caution!

· Please consult the Safety Data Sheet (SDS) of reagents and solvents used with the column and understand their proper handling methods to prevent potential health hazards or death from occurring.

· Please wear appropriate personal protective equipment such as lab goggles and gloves when handling organic solvents and acid and alkaline reagents. Avoid any direct physical contact to prevent chemical injuries.

Before Using the Column

(1) Please visually inspect the column package and the column surface for any damage.

(2) Please check the product name and serial number (Serial no. or S/N) written on the column package and adhesive label on the column body.

(3) Please download the Certificate of Analysis (CoA) for the purchased product. The CoA can be downloaded from Shodex website (https://www.shodex.com/download/). You will be asked to enter the serial number.

1. Introduction

Thank you for purchasing the Shodex product. Shodex IEC SP-FT 4A is a strong cation exchange column filled with non-porous polymer-basdfed gel modified with sulfopropyl group. It is an ideal column for analyzing various proteins, peptides, and components of nucleic acids as it has a low adsorption tendency towards hydrophobic compounds. The polymer-based gel allows its use at a wide pH range condition.

2. Column Components

Please refer to the Shodex website: https://www.shodex.com/en/da/07.html

3. Column Specifications

Product Code	Product Name	Column Size (mm)		Particle Size (μm)	Theoretical Plate Number (Per Column)	Ion Exchange Capacity (meq/g)
		I.D.	Length			
F6113100	**IEC SP-FT 4A**	4.6	10	2.7	≥ 20,000	0.2

Base Material : Non-porous particles of polyhydroxymethacrylate modified with sulfopropyl group

Column Housing : PEEK

Screw Type : Internally-threaded type No.10-32 UNF

Shipping Solvent : 20 mM MES* Buffer (pH5.6)

*2-(N-Morpholino)ethanesulfonic acid

4. Usable Conditions

Product Name	Flow Rate (mL/min)		Maximum Pressure (MPa) (System Pressure*)	pH Range	Temperature Range (°C)
	Recommended	Maximum			
IEC SP-FT 4A	1.0 - 2.0	3.0	20	2 - 12	5 - 45

* Total pressure of column and HPLC system

Usable solvents are listed below.

(1) The standard eluents are buffers and aqueous solutions of salt such as sodium chloride, potassium chloride, sodium sulfate, and potassium sulfate. Salts may also be added to the buffers. The recommended total salt concentration ranges are 0.1 M to 1 M. Please keep total concentration of salts under 1.5 M.Select a buffer which has negative charge ion and has a large buffering capacity at the desired pH. A list of usable buffers are given below.

pH Range	Buffer
3.8 - 4.3	Sodium Formate
4.3 - 4.8	Sodium Succinate
4.8 - 5.2	Sodium Acetate
5.0 - 6.0	Sodium Malonate
5.5 - 7.0	2-(N-morpholino)ethanesulfonic acid (MES)
6.7 - 7.6	Sodium Phosphate
6.8 - 8.2	2-[4-(2-Hydroxyethyl)-1-piperazinyl]-ethanesulfonic acid (HEPES)
7.7 - 9.1	N,N-Bis(2-hydroxyethyl) glycine (BICINE)
8.6 - 10.0	2-(cyclohexylamino)ethanesulfonic acid (CHES)

(2) Up to 20 % (v/v) acetonitrile can be added.

Attention!

· Use the column within above stated flow rate, pressure, and temperature ranges. Using the column outside the given range may damage the column and lower its performance.

· When using a mixture of buffer (or aqueous solution of salt) and organic solvent, make sure there is no precipitation of salt.

· When using highly corrosive salts such as sodium chloride, wash out the salts at the end of analysis. The metal parts of the devices and/or the columns may rust.

· Column pressure is influenced by the eluent composition, flow rate, and column temperature. When changing the eluent compositions, adjust the flow rate and column temperature so that the column pressure remains below the usable maximum pressure.

5. Eluent Preparation

(1) Degas the eluent fully to prevent the formation of air bubbles.

(2) Presence of small debris or insoluble substances may result in deterioration of columns and/or they may appear as noise on chromatograms. Filter the eluent with a 0.45-μm disposable filter to prevent the problems from occurring.

Attention!

· Whenever water is required, use ultra-pure water freshly generated by a water purification system or water from a newly opened HPLC grade distilled water bottle. Use of HPLC grade organic solvents of guaranteed quality, which can be used without problems in HPLC is recommended. If organic solvents with different grades are used together, make sure that their qualities are all suitable for the analysis prior to the use. Solvents left in opened bottles for a long time should not be used. The content may have been changed, absorbed moisture, or has been contaminated.

· Always use freshly prepared solvents. Solvents stored for a long time may have changed their compositions and may influence elution patterns and/or damage the column.

Note

· Use of an on-line degasser is recommended.

6. Sample Preparation

(1) If possible, use the eluent for analysis to dissolve or dilute samples. If this is difficult, use a solvent which has a composition that is as close as possible to the eluent composition and which fully dissolves or dilutes the sample. For gradient elution, samples are recommended to be dissolved or diluted using the eluent used at the beginning of the gradient method.

(2) Filter diluted sample solutions using disposable 0.45-μm filters to prevent the column from clogging or deteriorating.

(3) Suggested injection volume is less than 5 μL per column.

(4) When a sample contains lipids, remove them prior to the sample injection.

Attention!

· When a sample is dissolved in a solvent other than the eluent and if the sample matrix contains components which do not dissolve in the eluent fully, precipitates may form and clog the column.

· Full column performance may not be achieved if a sample concentration is higher or sample injection volume is larger than it should be. It may lead to abnormal peak shapes, poor separations, and/or low reproducibility. In such cases, please adjust the sample concentration and/or the injection volume.

7. Column Usage Procedure

7.1 HPLC System Preparation

Wash the entire HPLC system prior to column installation, including all flow-lines and sample loop by switching the valves, and then replace the washing solution with the eluent to be used. If desired new eluent has low miscibility/solubility to the eluent of previous analysis, first use the eluent that is miscible/soluble to both eluents, and then replace it with the desired eluent.

Attention!

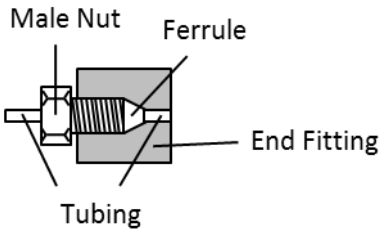
· If the eluent left in the HPLC system is not compatible with the column to be used, it may damage the column.

· A drastic change in the eluent compositions may remove substances adsorbed on the HPLC system and they may enter and deteriorate the column.

7.2 Column Installation

(1) Connect the column to HPLC system by following the “flow direction arrow” (➞) indicated on the column adhesive label.

(2) Make sure to insert the tubing all the way to the end fitting and secure it with the male nut. It is important that there is no extra space between the tubing and the column side of the end fitting. Presence of an extra space will let the sample to spread out and may result in wide peaks.



(3) Set the initial flow rate at less than 0.5 mL/min and start the system. If the column is to be heated during the analysis, keep the low flow rate until the column temperature reaches to the set temperature, and then gradually increase the flow rate to the desired temperature.

Caution!

· Verify that there is no solvent leak. The solvent leak may cause electronic leakage, rust, and/or chemical injury.

Attention!

· Make sure not to let air bubbles enter the column while installing the column. The air bubbles may damage the column.

· When restarting the system after column installation or after holding the eluent flow, start the system at less than 0.5 mL/min flow rate. A rapid increase in pressure can damage the column.

· If the column was heated during the analysis, lower the flow rate to less than 0.5 mL/min at the end of analysis. Then, turn off the column oven to let the column temperature returns to room temperature before stopping the pump. This is to prevent creating an empty space in the column, which deteriorates the column. Since if the pump was stopped while the eluent inside the column is still hot, the eluent volume decreases and creates an empty space when the eluent temperature decreases.

· Analytes are retained for very short time when using an ultra-rapid analysis and this makes their peak shapes sharp. Slow detector response results in broader peaks and this may appear as bad peak separations than actual. Please optimize the detector response setting by checking the peak separations. Also, slow data-processor sampling rate results in less data point collection and this may appear as bad peak shapes. Please set the data-processor’s sampling rate to obtain more than 20 data points for each peak width.

Note

· It is recommended to set the pump limiter to avoid exceeding the maximum pressure.

7.3 Solvent Exchange

(1) Check miscibility/solubility of the desired new solvent and the solvent currently filled in the column.

(2) When replacing the current solvent with a solvent with low miscibility/solubility to the current solvent, first use a solvent that is miscible/soluble to both solvents, and then replace it with the new solvent.

(3) When using a gradient method, changes in the eluent compositions may increase the column backpressure. Adjust the flow rate and column temperature so that the column backpressure remains below the usable maximum pressure throughout the analysis.

7.4 Column Cleaning

Problems in peak shapes and elution time changes or elevated column pressure are often caused by the deposition of insoluble or adsorbing components from the sample/flow-line inside the column. These problems may be resolved by cleaning the column. In case multiple number of analytical columns are used together, wash them separately. During the column cleaning, disconnect the detector and collect the washing solution directly from the column outlet into a waste container (i.e., do not let the solution go through the detector).

If the column performance does not improve (recover) after performing the column cleaning, please replace the column with a new one.

<Cleaning method>

Follow below cleaning steps for adsorbing components. Set the flow rate at less than 0.5 mL/min. Recommended solvent volume to introduce is 5 to 10 times of the column volume.

Method 1: Adsorption of proteins

Introduce an eluent with higher salt concentration (about 1 M) or 0.1 % nonionic surfactants.

Method 2: Adsorption of hydrophobic compounds

Introduce a mixture of solvent containing 50-mM eluent (buffer or aqueous salt solution) and 10 to 20 % (v/v) acetonitrile.

Attention! ·Keeping the washing solution in the column for a long time will lead to column deterioration. Please replace thewashing solution with the eluent immediately after cleaning.

8. Column Storage

Remove the column from HPLC system after replacing the in-column solvent with the initial shipping solvent. Securely tighten the end caps and store the column at a location with stable temperature (a cool and dark space is recommended). Refer to section 7.3 Solvent Exchange for how to replace the eluent.

Attention! · Never allow inside the column to dry. It can damage the column.

9. Column Inspection

Please refer to the inspection method described in the CoA. At Shodex, “half width method” is adopted for the calculation of plate count and “asymmetry factor (Fas)” is adopted for the calculation of peak symmetry. Please refer to the Shodex website for the detail: https://www.shodex.com/en/da/07.html

Attention! · Plate count and Fas values change significantly depend on samples and/or analysis conditions being used. To check the initial column condition, please make sure to use the same sample and the analysis condition mentioned in the CoA.

10. Additional Warnings

(1) Do not remove end fittings.

(2) Do not make a strong impact on the column. Do not drop or hit the column on a hard surface.

(3) Please follow a proper waste disposal method specified by your local regulations.

Please refer to the Shodex website (https://www.shodex.com/) for product details and their applications. For additional assistance, contact the distributor from whom you purchased the column or contact your regional Shodex support office (https://www.shodex.com/en/support_office/list).