Operation Manual

for

Shodex AFpak Series

For the longest possible service life of the column, please carefully observe the instructions in this manual.

Manufacturer:SHOWA DENKO K.K., Shodex(Separation & HPLC)Group

1. Introduction

The packed columns of the Shodex AFpak series are designed for high performance in affinity chromatography. The packing is prepared by putting a ligand in a covalent bond through the medium of spacers with the matrixes consisting of hydrophilic, highly porous polymer beads. The matrixes are chemically and physically so stable that the column can be used under the conditions that suit the ligand.

2. Specifications

Table 1 shows the specifications of Shodex AFpak series.
End fittings: Internally-threaded type No.10-32 UNF

3. Eluent

Generally speaking, adsorption of target substances comes first in affinity chromatography, then the substances are eluted by changing the composition of the eluent, usually in the following way:

1) Increasing the salt concentration in the eluent.
2) Changing the pH of the eluent.
   CAUTION
   The pH must remain in the 2 - 10 range if the ligand and the target substance are stable.
3) Mixing an organic solvent into it.
   NOTE
   Isopropyl alcohol or ethylene glycol is usually mixed in a quantity of 10 % maximum.
4) Mixing in a low-molecular-weight substance that interacts with the ligand.

Table 1 Specifications of Shodex AFpak series

(1-A)

Grade	Ligand	Column size(mm)	In-column solvent
AAB-894	Aminobenz amidine	8φ x 50	a
AAF-894	Acriflavine	8φ x 50	b
ABA-894K ABA-894KL	Bovine serum albumin	8φ x 50 8φ x 150	c
ABT-894	Biotin	8φ x 50	d
ACA-894	Concanavalin A	8φ x 50	e
ACB-894	Cibacron Blue	8φ x 50	f
ADS-894	Dextran sulfate	8φ x 50	g
AED-894	Ethylenediamine diacetic acid	8φ x 50	h
AGA-894	N-Acetyl glucosamine	8φ x 50	i
AGE-894	Gelatin	8φ x 50	c
AHR-894	Heparin	8φ x 50	j
AIA-894	Iminodiacetic acid	8φ x 50	h
AOV-894	Ovomucoid	8φ x 50	a
APA-894	Protein A	8φ x 50	k
APB-894 APB-894L	Aminophenyl boronic acid	8φ x 50 8φ x 100	l
APH-894	Phenyl alanine	8φ x 50	m
ARC-894	RCA-1	8φ x 50	n
AST-894	Soybean trypsin inhibiter	8φ x 50	a
AWG-894	Wheat germ agglutinin	8φ x 50	o
AAM-894	Adenosine 5'-monophosphate	8φ x 50	p
AAP-894	Aprotinin	8φ x 50	p
AAV-894	Avidin	8φ x 50	p
AGT-894	Glutathione	8φ x 50	p

(1-B)

Grade	Ligand	Column size(mm)	In-column solvent
ALC-894	Lens culinaris agglutinin	8ϕ x 50	q
ALS-894	Lysine	8ϕ x 50	p
ANA-894	Nicotinamide adenine dinucleotide	8ϕ x 50	p
APD-894	Procion red	8ϕ x 50	p
APE-894	Phosphoryl-ethanolamine	8ϕ x 50	p
APG-894	Protein G	8ϕ x 50	p
APR-894	Protamine	8ϕ x 50	p
APS-894	Pepstatin	8ϕ x 50	p

In-column solvent

a: 0.1M Sodium acetate buffer 0.5M NaCl 0.02% NaN_3 pH 7.0	k: 0.1M Sodium phosphate buffer 0.5M NaCl 0.02% NaN_3 pH 7.0
b: 0.1M Ethyl morpholine - Acetic acid buffer 0.02% NaN_3 pH 7.0	l: 0.01M Sodium phosphate buffer 0.1M NaCl 0.02% NaN_3 pH 7.0
c: 0.05M Tris-HCl buffer 0.15M NaCl 0.02% NaN_3 pH 7.4	m: 0.02M Sodium phosphate buffer 0.02% NaN_3 pH 6.0
d: 0.01M Sodium acetate buffer 0.02% NaN3 pH 6.5	n: 0.1M Sodium phosphate buffer 0.15M NaCl 0.2M Galactose 0.02% NaN_3 pH 7.4
e: 0.05M Tris-HCl buffer 0.15M NaCl 0.5mM $MnCl_2$,$CaCl_2$ 0.02% NaN_3 pH 7.4	o: 0.1M Tris-HCl buffer 0.15M NaCl 0.2M N-Acetyl glucosamine 0.02% NaN_3 pH 7.4
f: 0.1M Potassium phosphate buffer 0.02% NaN_3 pH 5.0	p: 0.01M Sodium phosphate buffer 0.15M NaCl 0.02% NaN_3 pH 7.4
g: 0.05M Sodium phosphate buffer 0.02% NaN_3 pH 7.4	q: 0.05M Tris-HCl buffer 0.1M NaCl 1mM $MnCl_2$,$CaCl_2$ 0.2M Glucose 0.01% Merthiolate pH 7.2
h: 0.05M Ethyl morpholine - Acetic acid buffer 0.02% NaN_3 pH 6.0	
i: 0.01M Tris-HCl buffer 0.02% NaN_3 pH 8.0	
j: 0.01M Tris-HCl buffer 0.01M NaCl 0.02% NaN_3 pH 7.4	

4. Filtering and degassing

Filter and degas the eluent as required.
Use of solvent degassing devices of the Shodex DEGAS KT series is recomended.

5. Column mounting

The column is equilibrated with the solvent contained in it prior to delivery to the user. See Table 1 for the in-column solvent.
Observe the following instructions in mounting the column.

1) Before mounting the column on the liquid chromatograph, thoroughly replace the solvent in the chromatograph with the one to be used as the eluent.
2) Set the flow rate at 3 ml/min maximum.
3) Mount the column so that of to faces the flow mark on the column downstream.

CAUTION

Utmost care must be exercised not to let air enter the column in the mounting process.

4) In eluting the gradient, take the following steps before starting separation to equilibrate the column.
   a) Pass 10-30 ml of buffer B (eluting solvent) through the column.
   b) Pass 10-30 ml of buffer A (adsorption solvent) thruogh the column.
5) Keep the column temperature constant as required.

CAUTION

The column temperature must be in the range of 5 to 40 °C.

6. Pretreatment of specimen

1) Dissolve the specimen in, if possible, the solvent to be used as the eluent. In eluting the gradient dissolve it in the initial eluent.
2) Pass the specimen through a 0.45 μm membrane filter to remove insoluble substances.

NOTE

Use of the disporsable filter unit Shodex DT ED-03, ED-13 or ED-25 is recommended.

7. Dismounting and storage

1) If the column is heated for separation, set the flow rate at 0.5 ml/min and stop heating. Keep the eluent flowing through the column until it cools down to room temperature.

CAUTION

Do not dismount the column before it has cooled to room temperature; otherwise, air will be drawn in the column during the dismounting process to deteriorate its performance.

2) Stop the pump and, if the column is to be reused on the following day, leave it on the chromatograph.
3) In case the column is not to be reused for some time, replace the eluent with a solvent containing substances such as 0.02 % sodium azide or 0.01 % Merthiolate, blank off both ends of the column and store it in a place where temperature is maintained at 4 °C.

CAUTION

If the column temperature goes down to 0 °C or below, the column will freeze, causing deterioration of its parformance.

8. Troubleshooting

Table 2 below shows troubles likely to occur during use of the column and the corrective actions to be taken.

After taking the corrective actions given in the table, check the column resolution. The column's performance can sometimes be restored.

Please note that removal of the end fittings will allow air or other extraneous matter to enter the column. therby probably further deteriorating its performance.

Table 2 Troubleshooting

Trouble	Cause	Corrective action
Column pressure increase	Plugged end fitting	1) Reverse the column on the chromatograph and the eluent through it at the rate of 0.5ml/min for 1 hour. 2) Replace the end fitting.
	Inclusion of extraneous substances in packings	Irreparable
Rapid deterioration of resolution	Void produced in the upstream end of column	Irreparable
	Liquid flow disturbance caused by extraneous matter cologging end fitting	Remove and wash end fitting in ultrasonic bath.
	Accumulation of adsorbed substances	Reverse the column on chromatograph and pass a suitable eluent through the column at the rate of 0.5ml/min and the resolving capacity may sometimes be recovered.
No elution of specimen	Specimen adsorbed	Change the eluting condition.
	Mulfunctioning detector	Check the detector.

9. Warranty

1) SHOWA DENKO shall replace any Shodex column,

① If found damaged at the time of delivery.

② If the separation obtained by the purchaser is significantly inferiorer to the one given in the inspection sheet attached to the column.

Claims must be filed with SHOWA DENKO within 10 days following delivery.

2) The following shall not be subject to warranty.

① Service life

② Deterioration of column performance resulting from inproper handling