228-10285B
April 2020

SHIMADZU

Shimadzu Packed Column for HPLC

Shim-pack™ DIOL Series

Instruction Manual

■ INTRODUCTION

Shim-pack DIOL columns are specifically designed for gel filtration chromatography of biological macro-molecules. The columns are packed with chemically bonded hydrophilic silica particles having controlled pore size distribution. Surface silanol groups of these materials are chemically modified with a hydrophilic polymer to eliminate the irreversible adsorption of proteins caused by acidic silanol groups, and consequently, a mixture of proteins is separated with high recovery, Since the packing materials are spherical, having an average particle diameter of 5 μm, these columns exhibit high efficiency.

■ OPERATING CARE

- Use the supplied tubing connectors for column connection. (See the section on Column Connection)
- Excessive tubing dead volume results in an apparent reduction in column efficiency. An appropriate length of tubing (1.6 mm O.D. × 0.3 mm I.D.) is from 50 to 100 mm.
- Observe the flow direction indicated on the column.
- Before attaching the column inlet to the appropriate port of the injector system, wash the flow line thoroughly with distilled water and water-miscible organic solvent, e.g. methanol or 2-propanol. It is recommended that a pre-column (P/N 228-16367-91) be placed between the HPLC pumping system and the sample injector in order to protect the analytical column from clogging.
- Maximum usable pressure is 20 MPa. Lower pressures result in longer lifetime at high efficiency.
- To protect the chemically modified silica surface, do not use solutions of lower than pH 2 or higher than pH 8.
- Do not subject to shock such as form dropping, etc.
- Do not exceed an operating temperature of 50 ℃.

■ COLUMN CONNECTION

- The column is connected with the following parts. Endure that the fittings are connected properly to avoid creating dead volume between the tubing and the column interface.

Item Name	P/N	Comments
Ferrule 1.6F 316L	228-16000-10	1/pkg
Male nut 1.6MN	228-16001	1/pkg

- Observe the appropriate connection method as illustrated in the Figure.

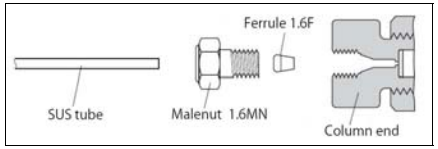

Fig.1 column connection

- Do not overtighten as this will result in damage to the fittings.

■COLUMN TYPES

The following types of DIOL columns are available. Select the type appropriate to your application.

Column	Dimension	Part No.
DIOL-150	7.9 mm I.D. × 250 mm	228-14775-91
	7.9 mm I.D. × 500 mm	228-14775-92
DIOL-300	7.9 mm I.D. × 250 mm	228-14776-91
	7.9 mm I.D. × 500 mm	228-14776-92

If you need a preparative column, contact your Shimadzu sales representative.

■COLUMN PERFORMANCE

To check the batch-to-batch reproducibility, each batch of DIOL materials is tested for protein separation. Typical chromatograms are shown in Figures 2 and 3.

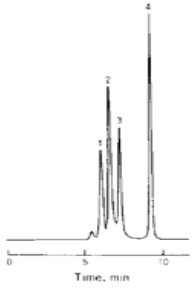

Fig.2 Typical chromatogram of a mixture of proteins on DIOL-150

OPERATING CONDITIONS
Column : Shim-pack DIOL-150 , 7.9 mm I.D. × 250 mm
Mobile Phase : 0.2 M sodium sulfate in 0.01 M phosphate buffer, pH7.0
Flow Rate : 1.0 mL/min
Temperature : 22 ℃
Detector : UV 280 nm
PEAK IDENTITY
1. Albumin; bovine serum
2. β-Lactoglobulin; cow's milk extract
3. Myoglobin; equine skeletal muscle extract
4. Glycyl-L-tyrosine

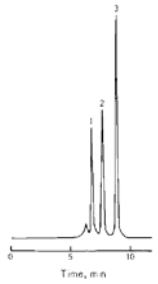

Fig.3 Typical chromatogram of a mixture of proteins on DIOL-300

OPERATING CONDITIONS
Column : Shim-pack DIOL-300 , 7.9 mm I.D. × 250 mm
Mobile Phase : 0.2 M sodium sulfate in 0.01 M phosphate buffer, pH7.0
Flow Rate : 1.0 mL /min
Temperature : 22 ℃
Detector : UV 280 nm
PEAK IDENTITY
1. Albumin; bovine serum
2. Myoglobin; equine skeletal muscle extract
3. Glycyl-L-tyrosine

■SELECTION OF MOBILE PHASE

- Typical buffers used for classical gel filtration chro-matography are acceptable for the DIOL columns, and the packing material is compatible with buffers, detergents and organic solvents. A typical buffer used is 0.1 ~ 0.2 M phosphate buffer, pH 7.0.
- Filter the mobile phase through a membrane filter of 0.2 ~ 0.45 μm pore size.
- Samples should be filtered through a membrane filter. Careful handling of the mobile phase and sample solutions increases the lifetime of the column.
- Solvents, salts and buffers should be of HPLC or analytical grade.
- Recommended flow rates are from 0.5 ~ 1.2 mL/min for best resolution.

■COLUMN WASHING

An DIOL column can be washed with 0.1% trifluoroacetic acid (TFA) and 2-propanol in case increased back pressure or loss of resolution is observed.

- For washing the column, flow rates up to 0.5 mL/min are recommended.
- If an increased back pressure is observed, check the back pressure without the column and make sure than the flow line of the HPLC system is not clogged. In case of a clogged flow line, take appropriate actions (such as replacing the line filter or connecting tubes).
- If an increased pressure across the column itself is observed, wash the column according to the wash procedure above.

■COLUMN STORAGE

- Do not store the column in a mobile phase which contains halides.
- Be careful to avoid potential bacterial growth. It is recommended that the storage liquid having antimicrobial action.
- Column should be stored at 4 to 35 ℃.
- Prefill solvent in the column when shipped: same as described in the column performance report.

■TECHNICAL SUPPORT

Shim-pack DIOL columns are manufactured, inspected, packaged and shipped under strict standards of quality control. Should you find any defect in performance, please contact your local Shimadzu representative, who will ensure your complete satisfaction.
We regret that we cannot guarantee the lifetime of columns, also that we cannot accept any claim when performance has deteriorated due to noncompliance with the operation procedures elucidated above, or as a result of normal aging.

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