

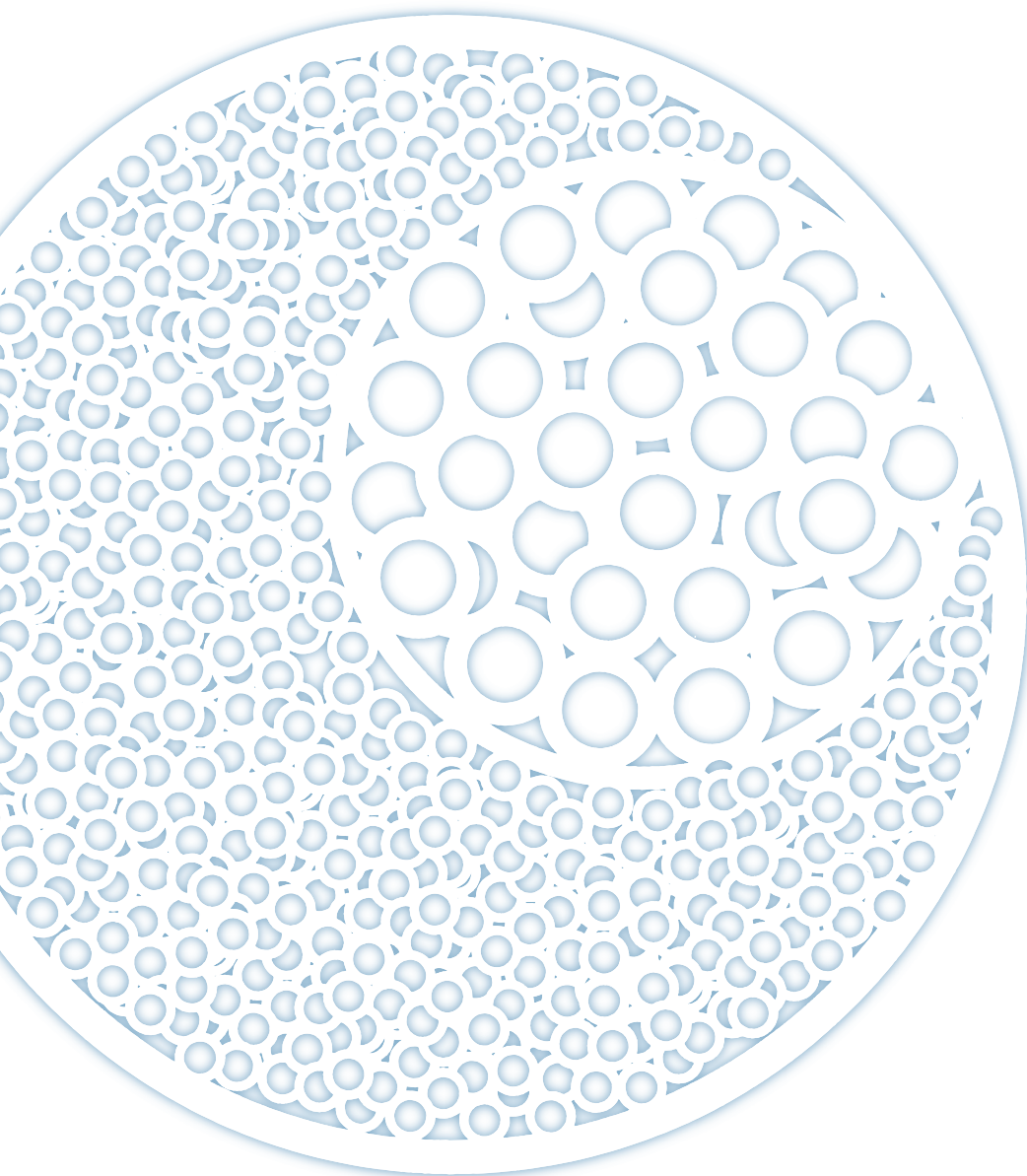


TOSOH

# **MiniChrom Columns for TOYOPEARL® and TSKgel®**

Pre-packed columns for resin screening and methods development

*Instruction Manual*



**TOSOH BIOSCIENCE**

## Introduction

MiniChrom columns for TOYOPEARL and TSKgel are part of the method development platform available from Tosoh Bioscience. The columns (5 mL, 8 mm ID × 10 cm) are pre-packed with a range of TOYOPEARL and TSKgel chromatography resins. They are designed for method optimization, parameter screening and/or small scale purifications.

The 5 mL MiniChrom columns are the ideal tool to further optimize a purification method and/or to confirm operational parameters after having selected a resin for a certain purification task by resin screening, e.g. with ToyoScreen® cartridges on conventional LC systems or by high throughput screening using RoboColumns® on robotic workstations. Each column is individually flow-packed under optimum conditions for each resin to give an accurate representation of performance at large scale.

The columns can be connected directly to any laboratory liquid chromatography system via standard HPLC connectors (M10-32 for 1/16" tubing) and are ready for equilibration in the buffer of choice. Two columns can be connected in series to increase the column height in order to model real conditions in pilot scale or for scale-down experiments.

## Product Attributes

- Pre-packed with select TOYOPEARL and TSKgel resins
- Ready-to-use 5 mL columns
- Easily connects to common laboratory liquid chromatography systems
- Generates reliable and reproducible results
- Ideal for parameter screening and method optimization

## Product Description

### Materials

MiniChrom columns are made of biocompatible polymers, mainly high-density polyethylene (column tube, lock nut, filter adapter). The frits are made from a combination of porous polypropylene/polyethylene material. *Figure 1* shows the parts of a MiniChrom 8 mm ID × 10 cm column. The upper & lower filter plates, together with the frits, are internal parts.

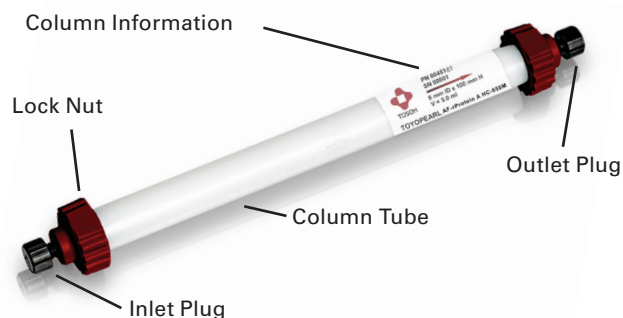
### Connectability

MiniChrom columns are delivered ready to use, packed with TOYOPEARL or TSKgel chromatography media. Columns may be connected directly to most liquid chromatography systems or workstations.

MiniChrom columns may be reused as long as the resin remains in acceptable condition, depending largely upon the level of care taken, the cleaning procedures used and the tolerance of the resin to user conditions. The columns are not designed to be repacked.

The properties of MiniChrom 8 mm ID × 10 cm columns are summarized in *Table 1*.

*Figure 1: MiniChrom column*



*Table 1: Properties of MiniChrom columns*

Property	Specification
Interior dimensions	8 mm ID × 10 cm bed height
Exterior dimensions	10 mm ID × 16.4 cm length
Cross sectional area	0.5 cm <sup>2</sup>
Column volume	5.0 mL
Frit pore size	14 µm
Max column pressure <sup>1</sup>	3.0 MPa
Max resin pressure	0.3 MPa – TOYOPEARL 2.0 MPa – TSKgel
Connectors	Standard HPLC (10-32 for 1/16" capillary tubing)
Shipping buffer	Unless otherwise indicated: 20% EtOH or a mixture of 20% EtOH and 0.15 mol/L NaCl (IEC and HIC resins)
Chemical stability <sup>2</sup>	Columns are tolerant to aqueous buffers and salt solutions, 1 mol/L alkaline solutions, 1 mol/L non-oxidizing mineral acids, 8 mol/L urea, 6 mol/L guanidine hydrochloride, non-halogenated organic solvents and detergents. They are <u>not</u> compatible with strong oxidants and halogenated solvents.

<sup>1</sup> This is dependent on the resin packed in the column.

<sup>2</sup> The chemical stability refers to the column hardware only. The individual packed chromatography media may have different stability. To avoid damage to the chromatography media, please refer to the relevant resin data.

**Note:** The use of multivalent salts in the mobile phase with TOYOPEARL NH<sub>2</sub>-750F may have adverse effects on the binding capacity of this resin. Please be sure to only use monovalent salts with TOYOPEARL NH<sub>2</sub>-750F.

## Instructions for Use

Install the column following the recommended flow direction as indicated by the arrow on the column label. For best results and increased column life, samples and buffers should be degassed and filtered through a 0.45 µm filter.

A sudden pressure change may impair the performance of the column. Therefore, slowly step-up the mobile phase flow rate until it reaches the desired value.

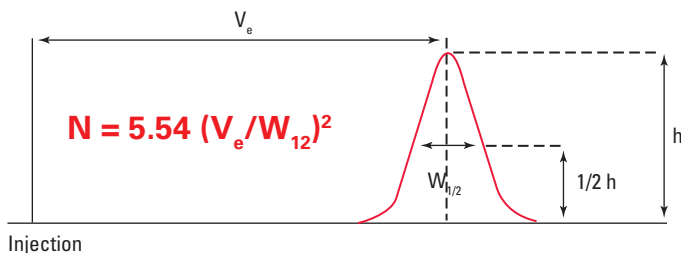
Before loading the column for the first time, the column needs to be equilibrated with at least 10 column volumes (CV) of the respective loading buffer. For washing and equilibration, a flow rate of 5 mL/min (2.5 mL/min for TOYOPEARL AF-rProtein A HC-650F) may be used as a suggested flow rate. These flow rates correspond to a linear velocity of 600 cm/hr and 300 cm/hr, respectively.

Buffer composition and gradient design will depend on the resin being used. Please refer to the *Instruction Manual for TOYOPEARL and TSKgel PW-type Resins* (IM02) and/or the respective Operating Conditions and Specifications datasheet of the resin for more information about recommended eluents, storage and cleaning solutions.

## Performance Testing

The chromatographic performance of MiniChrom columns should be checked initially and at regular intervals by determination of plate number and asymmetry factor. The theoretical plate number, *Figure 2*, ( $N$ , as number per meter) and the asymmetry factor ( $A_s$ ), *Figure 3*, are calculated according to the equations below.

Figure 2: Calculation of the number of Theoretical Plates and HETP



Where:

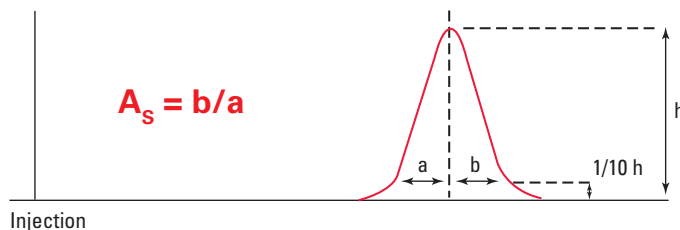
- $N$  = number of theoretical plates
- $V_e$  = elution volume or retention time (mL, sec, or cm)
- $h$  = peak height
- $W_{1/2}$  = peak width at 1/2 peak height (mL, sec, or cm)

$$H = L/N$$

Where:

- $H$  = height equivalent of a theoretical plate
- $L$  = length of the column
- $N$  = number of theoretical plates

Figure 3: Calculation of peak asymmetry factor



Where:

- $A_s$  = peak asymmetry factor
- $b$  = distance from the point at peak midpoint to the trailing edge (measured at 10% of peak height)
- $a$  = distance from the leading edge of peak to the midpoint (measured at 10% of peak height)

The functional test for columns packed with chromatography media incompatible with acetone, e.g. protein A resins, should be performed with an alternative low molecular weight sample such as NaCl.

Please note: the *Instruction Manual for TOYOPEARL and TSKgel PW-type Resins*, as well as Operating Conditions and Specifications datasheets, can be downloaded from the Tosoh Bioscience LLC website: [www.separations.us.tosohbioscience.com](http://www.separations.us.tosohbioscience.com).

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