

SkillPak™ Columns 1 mL and 5 mL pre-packed



TOSOH BIOSCIENCE



NOMENCLATURE

What's in our names?

Tosoh Bioscience has the most comprehensive selection of process media resins, with a variety of pore and particle size combinations for several modes of chromatography. When it comes to naming our resins, we've got it down to a science (literally). Here's how you can identify the right resin for your purification process:

1. Resin Type

Tosoh Bioscience offers two base beads for our resin products: TOYOPEARL® and TSKgel®.

TOYOPEARL and TSKgel products are hydroxylated methacrylic polymer resins and are offered in many different pore sizes and particle diameters. The key differences between the two types are particle size availability, degree of crosslinking, dynamic binding capacity, and operating pressures. Since similarly functionalized TOYOPEARL and TSKgel resins have the same backbone polymer chemistry, the selectivity remains the same as you scale up or down.

5. Additional Abbreviations

Some of our products have additional features or need clarification about what type of product they are.

We use the following abbreviations to highlight these features:

HC	High Capacity
AR	Alkaline Resistant
MX	Mixed-Mode
AF	Affinity
Super	High Capacity Ion Exchanger
MegaCap	High Capacity Ion Exchanger for Capturing
GigaCap	Ultra High Capacity Ion Exchanger

Anion Excha
Catior Excha
Antibo Affinit
Mixed Mode

4.

2. Ligand

TOYOPEARL or TSKgel resins are available in the following modes of chromatography functionalized with these ligands:

Mode	Ligand
HIC	Ether, PPG, Phenyl, Butyl, Hexyl
Anion Exchange	DEAE, QAE, Q, NH ₂
Cation Exchange	CM, SP, Sulfate
Antibody Affinity	rProtein A, rProtein L
Affinity	Tresyl, Epoxy, Formyl, Amino, Chelate, Red, Heparin, Carboxy
Mixed- Mode	Tryptophan (Trp)

4. Particle Size

Particle size is typically denoted in the product name as letters or numbers denoting the grade.

3. Pore Size

TOYOPEARL or TSKgel resins are available in the following pore sizes:

Particle size of TOYOPEARL and TSKgel resins (µm)

Particle	SIZE OF TOYOPEARL and 15	Kgei resins (µm)	
Grade	TOYOPEARL	TOYOPEARL GigaCap	TSKgel
EC	200		
С	100 (SEC resins are 75)		
M	65 (MX-Trp is 75)	75	
F	45		
S	35 (SEC resins are 30)	35	
(30)			30
(20)			20

TOYOPEARL and TSKgel resin number key TOYOPEARL 550 resins HW-55 base resin 50 nm pore size TOYOPEARL 600 resins HW-60 base resin 75 nm pore size TOYOPEARL 650 resins HW-65 base resin 100 nm pore size

TOVOPEARL 750 regine

TOTOT EATTE 750 TCSIIIS	1144 75 6450 105111	> 100 IIIII pore 3120
TSKgel 3PW resin	PW-3000 base resin	25 nm pore size
TSKgel 5PW resin	PW-5000 base resin	100 nm pore size

HW-75 hase resin

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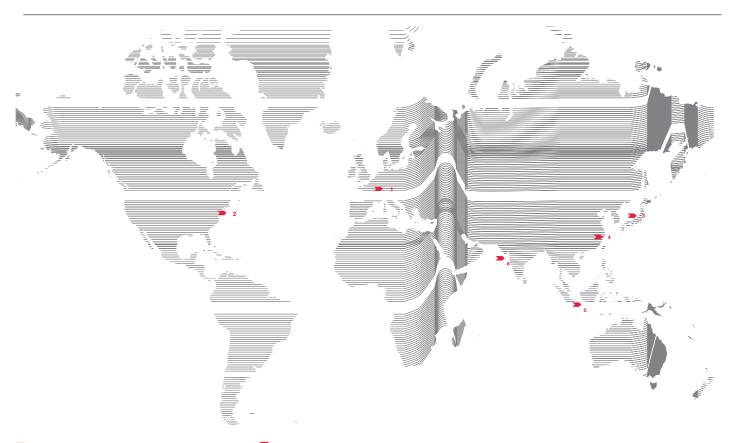
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TOSOH HISTORY

1936	Operation of Nanyo Manufacturing Complex begins
1979	Tosoh develops TOYOPEARL media
1983	First TOYOPEARL hydrophobic interaction (HIC) resin
1995	Tosoh Nanyo gel factory receives ISO9001
2007	TOYOPEARL GigaCap high capacity ion exchange series starts
2012	A second TOYOPEARL production site doubles manufacturing capacity
2012	First TOYOPEARL multimodal resin
2013	High capacity TOYOPEARL Protein A resin for antibody purification introduced
2014	TOSOH Bioscience GmbH celebrates its 25th anniversary
2016	First salt-tolerant TOYOPEARL ion exchanger
2016	TOYOPEARL® Sulfate-650F receives the TMM Innovation Award 2016
2017	High capacity TOYOPEARL Protein L resin for antibody purification introduced
2019	Completion of the third TOYOPEARL factory
2020	SkillPack pre-packed columns launched globally

Founding of Toyo Soda Manufacturing Co., Ltd.



INTRODUCTION

SkillPak 1 and 5 mL columns are designed for fast method development, resin screening or sample concentration. Pre-packed with TOYOPEARL, TSKgel or Ca**Pure-HA process chromatography media, these columns offer superior evaluation of biomolecules, such as monoclonal antibodies, proteins and oligonucleotides.

SkillPak 1 and 5 mL pre-packed columns are designed for your purification and separation tasks from platform design to pilot scale. These columns are ready to use upon receipt and show excellent physical strength and ideal flow characteristics for industrial downstream processing.

Tables 1 and 2 below list the properties and operation specifications of the SkillPak 1 and 5 mL columns. These columns guarantee optimal performance and can be operated with commonly used low or medium pressure liquid chromatography systems. They are reproducibly packed and take into account the varying compressibility of each resin. This provides an accurate representation of conditions found in full scale columns.

₹TABLE 1 -

SPECIFICATIONS OF SkillPak 1 mL COLUMN

Column dimension	7 mmID×2.5 cm bed height
Volume	1 mL
Maximum flow rate	4 mL/min (600 cm/hr)
Maximum operat- ing pressure	0.3 MPa
Connections	Standard fittings (10-32 for 1/16 inch capillary)
Shipping buffer	20% ethanol for TOYOPEARL and TSKgel [with the exception of 0.5 mol/L sodium citrate with 20% ethanol for TSKgel SP-5PW (20) and SuperQ-5PW (20)], 20 mmol/L sodium phosphate with 20% ethanol for Ca**Pure-HA

APPLICATIONS

Design of Experiments (DoE) is a systematic method to determine the effects of various factors that can influence the output results. It is a technique used to generate the required information with the minimum amount of experimentation. Initial experiments are required in order to define the ranges for variables. DoE can be used for parameter screening and optimization as well as for robustness testing.

It is important to consider processing variables, including environmental constraints, early in the development of a purification process. SkillPak column formats are ideal to use for parameter and method optimization and for robustness testing for the development of a new or replacement purification process.

■ TABLE 2

SPECIFICATIONS OF SkillPak 5 mL COLUMN

Column dimension	8mmID×10cm bed height
Volume	5 mL
Standrad flow rate	1.3 mL/min (150 cm/hr)
Maximum flow rate	5 mL/min (600 cm/hr) for TOYOPEARL M and C grade resins; 2.5 mL/min (300 cm/hr) for TSKgel, Ca**Pure-HA and TOYOPEARL S- and F-grade resins, including TOYOPEARL AF-rProtein A HC-650F and TOYOPEARL AF-rProtein L-650F
Maximum operat- ing pressure	0.3 MPa for TOYOPEARL resins, ≤0.4 MPa for TSKgel resins and Ca ⁺⁺ Pure-HA
Connections	Standard fittings (10-32 for 1/16 inch capillary)
Shipping buffer	20% ethanol for TOYOPEARL and TSKgel, 20 mmol/L sodium phosphate with 20% ethanol for Ca ⁺⁺ Pure-HA
Asymmetry factor (As) specifications	0.8-1.4 for TOYOPEARL and TSKgel, 0.8-2.6 for Ca ⁺⁺ Pure-HA

Method development for mAb purification: Determine elution pH for a mAb using SkillPak 1 mL and 5 mL columns

Finding the appropriate buffer and pH for mAb elution reduces the risk of increasing aggregation in the mAb sample. To identify optimized conditions for mAb binding and elution, a buffer-adjusted mAb containing hybridoma cell line supernatant was loaded onto a SkillPak 5 mL column pre-packed with TOYOPEARL AF-rProtein A HC-650F media.

CONDITIONS

TOYOPEARL AF-rProtein A HC-650F

Equilibration 0.1 mol/L sodium phosphate, buffer: 0.15 mol/L NaCl, pH7.3

Mobile phase A 25 mmol/L citrate (NaOH), pH4.5

(gradient):

Mobile phase B 25 mmol/L citrate (NaOH), pH3.0

(gradient):

Elution gradient: Linear from gradient buffer A to

100% B over 10CV

Flow (load): 0.25 mL/min (1 mL column);

1.5 mL/min (5 mL column)

1.0 mL/min (1 mL column);

(wash, gradient): 2.0 mL/min (5 mL column) using

ÄKTA™ avant 25

2mL of buffer-adjusted CHO cell culture Samples:

supernatant containing 4mg mAb

(1mL column);

14mL of buffer-adjusted CHO cell culture supernatant containing 20 mg mAb

(5 mL column)

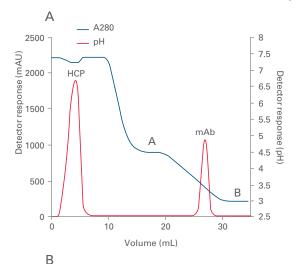


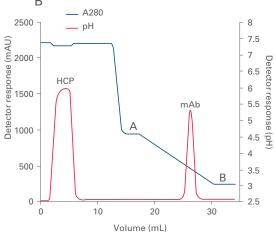


Figure 1, Panels A & B, demonstrate that a sharp mAb peak with elution max at pH 4.0 was obtained using a linear pH gradient. To maximize recovery, pH 3.5 was selected for step elution.

FIGURE 1

DETERMINATION OF ELUTION pH FOR mAb USING SkillPak 1 mL TOYOPEARL AF-rProtein A HC-650F COLUMN (PANEL A); SkillPak 5 mL TOYOPEARL AF-rProtein A HC-650F COLUMN (PANEL B)







Method development for mAb purification: capture of intact antibodies using SkillPak 5 mL column

340 mL of hybridoma cell line supernatant (titer ~0.03 g/L) was loaded on the SkillPak 5 mL column packed with TOYOPEARL AF-rProtein A HC 650F resin.

CONDITIONS

SkillPak 5 mL Column:

TOYOPEARL AF-rProtein A HC-650F

Equilibration buffer: 0.1 mol/L Na HPO /NaH PO ,/

0.15 mol/L NaCl, pH7.3

Post-loading 1st wash: equilibration buffer (5CV) Post-loading 2nd wash: 0.1 mol/L acetate (NaOH),

pH5.0 (5CV)

Elution: 0.1 mol/L acetate (NaOH),

pH3.5 (5CV)

Column strip: 0.1 mol/L acetic acid, pH2.9 (3CV) Column cleaning: 0.2 mol/L NaOH (3CV), 15 min hold Flow (load): 150 cm/hr (1.25 mL/min), 4 min

residence time

Flow (wash/elution): 240 cm/hr (2.0 mL/min)

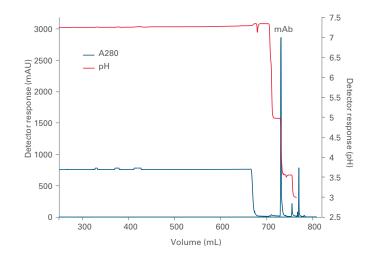
(ÄKTA avant 25)

Temperature: ambient (room temperature) Sample: 340 mL hybridoma cell culture supernatant (buffer-adjusted)

After a short wash at pH 5.0, a sharp and efficient elution peak was obtained at the start of the pH 3.5 elution (Figure 2). Total mAb recovery was 9.8 mg in the elution peak (4.8 mL).

FIGURE 2

CAPTURE OF INTACT mAb USING SkillPak 5 mL TOYOPEARL AF-rProtein A HC-650F COLUMN



Method development for mAb purification: mAb eluate polishing using SkillPak 1mL column

Eluate was prepared for a polishing step on a SkillPak 1 mL column packed with TOYOPEARL Sulfate-650F by adding four eluate volumes of 0.2mol/L acetate, pH5.0. Aggregate and other impurities in the protein A eluate were removed by using the SkillPak 1mL TOYOPEARL Sulfate-650F column. Figure 3 shows an efficient elution of mAb at a 0.35 mol/L NaCl step in the equilibration buffer. Impurities were removed by the polishing step as can be seen before and after the elution of the mAb peak.

CONDITIONS

SkillPak 1 mL TOYOPEARL Column:

Sulfate-650F

Equilibration buffer: 0.1 mol/L acetate (NaOH), pH5.0

Post-loading 1st wash: equilibration buffer (5CV)

Post-loading 2nd wash: 0.1 mol/L acetate (NaOH), 0.1 mol/L

NaCl, pH5.0 (10CV)

Elution: 0.1 mol/L acetate (NaOH),

0.35 mol/L NaCl, pH5.0 (10 CV)

Column strip: 0.1 mol/L acetate (NaOH),

1.0 mol/L NaCl, pH5.0 (7 CV)

Flow (all steps): 156cm/hr (1.0 mL/min) 4 min

residence time (AKTA avant 25)

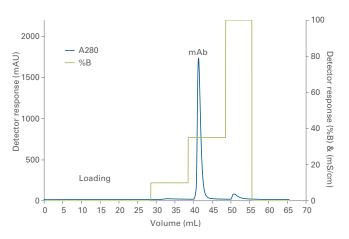
Temperature: ambient (room temperature)

Sample: 21 mL (0.41 mg total mAb) diluted

protein A eluate

FIGURE 3

POLISHING CHROMATOGRAPHY USING SkillPak 1 mL TOYOPEARL Sulfate-650F COLUMN



Column reproducibility: capture of intact mAb using SkillPak 5 mL column

A SkillPak 5 mL column prepacked with TOYOPEARL AF-rProtein A HC-650F resin was loaded with 23.5 mg of mAb feedstock. After 10 cycles the average recovery was 91% (average yield was 21.3 mg) as shown in the table below. Both pH max for elution and yield were highly consistent throughout the 10 cycles.

Reproducibility of SkillPak 5 mL column for capture of intact mAb

Run #	Retention @ peak max (mL)	Peak area (mL*mAU)	Peak height (mAU)	pH @ peak max	Eluate A280 (1:10 dil.)	Eluate (mL)	Yield (mg)
1	102.1	6327	1599	3.57	0.309	9.62	21.9
2	102.1	6335	1587	3.57	0.299	9.79	21.5
3	102.2	6351	1577	3.56	0.312	9.34	21.4
4	102.1	6358	1620	3.57	0.296	9.60	20.9
5	102.3	6308	1572	3.56	0.290	9.86	21.0
6	102.0	6310	1619	3.57	0.298	9.56	20.9
7	102.0	6300	1630	3.55	0.300	9.45	20.8
8	102.0	6294	1622	3.54	0.298	9.51	20.8
9	102.1	6309	1605	3.53	0.312	9.65	22.1
10	102.2	6291	1596	3.57	0.307	9.71	21.9
Ave.	102.1	6318	1603	3.56			21.3
St.Dev.	0.1	23	20	0.01			0.5

Method development for Fab purification: Capture of Fab antibodies using SkillPak 1mL column

Humanized IgG1 was digested using a papain enzyme to obtain Fab, (see procedure below). $100\,\mu\text{L}$ of the papain-digested IgG1 which contains Fab material was loaded onto a SkillPak 1mL column pre-packed with TOYOPEARL AF-rProtein L-650F resin.

3.5 g/L human lgG₁
50 mmol/L sodium phosphate buffer, pH 7.0
150 mmol/L sodium chloride
10 mmol/L l-cystein
10 mmol/L EDTA

25 °C 5h incubation

Add 1/10 volume of 0.3 mol/L N-Ethylmaleimide

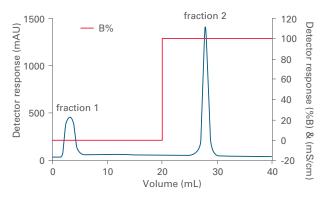
Fab to be loaded onto SkillPak 1 mL

TOYOPEARL AF-rProtein L-650F column

Figure 4 shows that Fab from the digested humanized IgG1 was successfully captured by the SkillPak 1 mL TOYOPEARL AF-rProtein L-650F column. Fab was eluted at approximately 28 minutes.

≅ FIGURF 4

CAPTURE OF Fab USING SkillPak 1 mL TOYOPEARL AF-rProtein L-650F COLUMN



Column: SkillPak 1 mL TOYOPEARL AF-rProtein L-650F

Flow rate: 0.5 mL/min

Mobile phase: A: 0.1 mol/L citrate buffer, pH 6.5

B: 0.1 mol/L citrate buffer, pH2.2

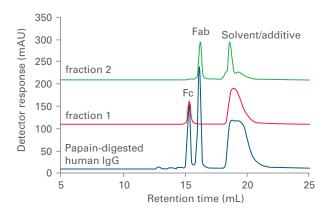
Detection: UV @ 280 nm

Sample: papain-digested human lgG1

The unbound peak (fraction 1) and the Fab peak (fraction 2) were captured and then analyzed using a (U)HPLC size exclusion chromatography column, TSKgel UP-SW3000. Figure 5 shows that only fraction 2 contained Fab material, as compared to the papain-digested IgG (reference material).

FIGURE 5

ANALYSIS OF CAPTURED Fab FROM THE DIGESTED $\lg G1$ USING TSKgel UP-SW300



Column: TSKgel UP-SW3000, 2 µm, 4.6 mm IDx30 cm L

Flow rate: 0.5 mL/mir

Mobile phase: 50 mmol/L phosphate buffer + 0.2 mol/NaCl, pH6.8

Detection: UV @ 280 nm

Samples: fractions of protein L chromatography

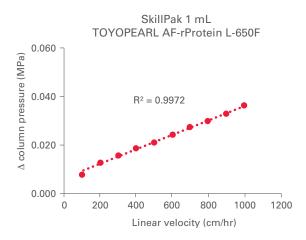
papain-digest of human IgG

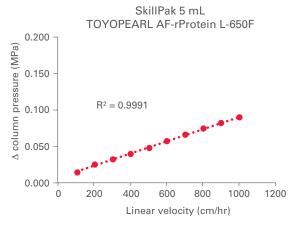


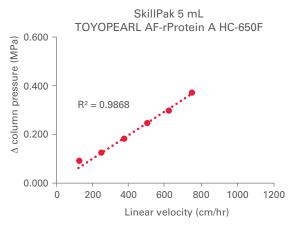
PACKING PERFORMANCE

SkillPak 1mL and 5mL columns exhibit excellent flow characteristics packed with specified process resins and are compatible with low to medium pressure chromatography systems. Small configurations reduce resin costs and save valuable samples while delivering similar performance to larger preparative process columns. The figures below demonstrate the superior packing performance for SkillPak 1 mL and 5 mL columns pre-packed with a variety of TOYOPEARL resins.

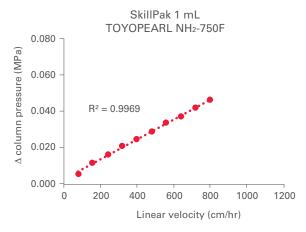
SkillPak 1 mL and 5 mL pre-packed with TOYOPEARL AF-rProtein L-650F and AF-rProtein A HC-650F affinity resins

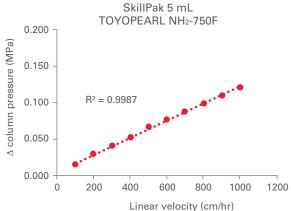


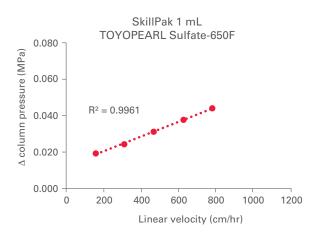


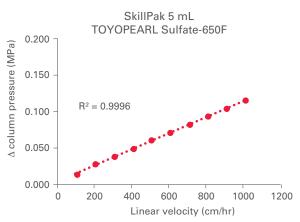


SkillPak 1 mL and 5 mL pre-packed with TOYOPEARL NH $_2$ -750F and Sulfate-650F ion exchange resins



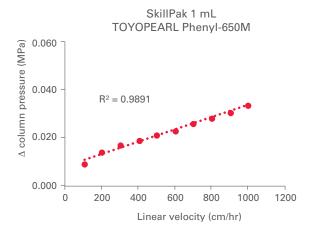


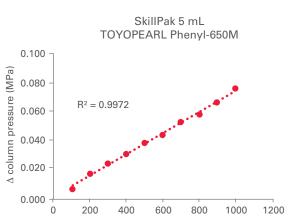




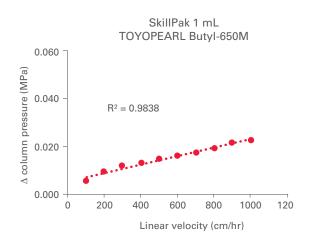


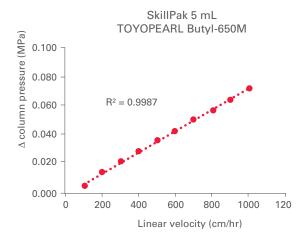
SkillPak 1 mL and 5 mL pre-packed with TOYOPEARL Phenyl-650M and Butyl-650M hydrophobic interaction chromatography resins





Linear velocity (cm/hr)



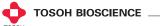






ORDERING INFORMATION: SkillPak 1 mL COLUMNS

Part #	Description
Affinity Chromatogr	aphy
0045200	SkillPak TOYOPEARL AF-rProtein L-650F 1 mL column
0045221	SkillPak TOYOPEARL AF-rProtein L-650F 1 mL columns (qty. 5)
0045201	SkillPak TOYOPEARL AF-rProtein A HC-650F 1 mL column
0045222	SkillPak TOYOPEARL AF-rProtein A HC-650F 1 mL columns (qty. 5)
0045202	SkillPak TOYOPEARL AF-Chelate-650M 1 mL column
Ion Exchange Chron	natography
0045203	SkillPak TOYOPEARL GigaCap Q-650M 1 mL columns (qty. 5)
0045204	SkillPak TOYOPEARL GigaCap S-650S 1 mL columns (qty. 5)
0045205	SkillPak TOYOPEARL Sulfate-650F 1 mL columns (qty. 5)
0045206	SkillPak TOYOPEARL SuperQ-650S 1 mL columns (qty. 5)
0045207	SkillPak TSKgel SP-5PW (20) 1 mL columns (qty. 5)
0045208	SkillPak TSKgel SuperQ-5PW (20) 1 mL columns (qty. 5)
0045209	SkillPak TOYOPEARL NH2-750F 1 mL columns (qty. 5)
0045210	SkillPak TOYOPEARL GigaCap DEAE-650M 1 mL columns (qty. 5)
0045211	SkillPak TOYOPEARL GigaCap CM-650M 1 mL columns (qty. 5)
0045212	SkillPak TOYOPEARL QAE-550C 1 mL columns (qty. 5)
0045213	SkillPak TOYOPEARL SP-550C 1 mL columns (qty. 5)
Hydrophobic Interac	ction Chromatography
0045214	SkillPak TOYOPEARL Butyl-600M 1 mL columns (qty. 5)
0045215	SkillPak TOYOPEARL Butyl-650M 1 mL columns (qty. 5)
0045216	SkillPak TOYOPEARL Phenyl-600M 1 mL columns (qty. 5)
0045217	SkillPak TOYOPEARL Phenyl-650M 1 mL columns (qty. 5)
0045218	SkillPak TOYOPEARL PPG-600M 1 mL columns (qty. 5)
0045219	SkillPak TOYOPEARL Hexyl-650C 1 mL columns (qty. 5)
0045220	SkillPak TOYOPEARL Ether-650M 1 mL columns (qty. 5)
Mixed Mode Chrom	atography
0045224	SkillPak TOYOPEARL MX-Trp-650M 1 mL columns (qty. 5)
Size Exclusion Chro	matography
0045223	SkillPak TOYOPEARL HW-40F 1 mL columns (qty. 5)
HA Chromatograph	У
0045225	SkillPak Ca ⁺⁺ Pure-HA 1 mL column
Column Libraries	
0045226	SkillPak Anion Exchange 1 mL column library, 1 mL × 2 each (TOYOPEARL GigaCap Q-650M, GigaCap DEAE-650M, NH ₂ -750F)
0045227	SkillPak Cation Exchange 1 mL column library, 1 mL × 2 each (TOYOPEARL GigaCap S-650S, GigaCap CM-650M, Sulfate-650F)
0045228	SkillPak Antibody 1 mL column library, 1 mL × 1 each (TOYOPEARL AF-rProtein A HC-650F, AF-rProtein L-650F, NH2-750F, Sulfate-650F, GigaCapQ-650M, GigaCapS-650S)
0045229	SkillPak mAb Platform 1 mL column library, 1 mL × 2 each (TOYOPEARL AF-rProtein A HC-650F, Sulfate-650F, NH2-750F)





ORDERING INFORMATION: SkillPak 1 mL COLUMNS, continued

Part #	Description
0045230	SkillPak Salt Tolerant 1 mL column library, 1 mL × 3 each (TOYOPEARL Sulfate-650F, NH2-750F)
0045231	SkillPak Mixed Mode 1 mL column library, 1 mL x 3 each (Ca ⁺⁺ Pure-HA, TOYOPEARL MX-Trp-650M)
0045232	SkillPak Best-in-Class 1 mL column library, 1 mL x 1 each (Ca ⁺⁺ Pure-HA, TOYOPEARL AF-rProtein A HC-650F, AF-rProtein L-650F, NH ₂ -750F, Sulfate-650F)
0045233	SkillPak HIC 1 mL column library, 1 mL × 1 each (TOYOPEARL Butyl-650M, Phenyl-650M, PPG-600M, Hexyl-650C, Ether-650M)
0045236	SkillPak HIC 1 mL column library, 1 mL × 2 each (TOYOPEARL Butyl-600M, Phenyl-600M, PPG-600M)



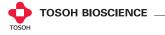


ORDERING INFORMATION: SkillPak 5 mL COLUMNS

Part #	Description
Affinity Chromatography	y
0045257	SkillPak TOYOPEARL AF-rProtein L-650F 5 mL column
0045258	SkillPak TOYOPEARL AF-rProtein A HC-650F 5 mL column
0045259	SkillPak TOYOPEARL AF-Chelate-650M 5 mL column
Ion Exchange Chromato	graphy
0045239	SkillPak TOYOPEARL GigaCap Q-650M 5 mL column
0045240	SkillPak TOYOPEARL GigaCap S-650S 5 mL column
0045241	SkillPak TOYOPEARL Sulfate-650F 5 mL column
0045242	SkillPak TOYOPEARL SuperQ-650S 5 mL column
0045243	SkillPak TSKgel SP-5PW (20) 5 mL column
0045244	SkillPak TSKgel SuperQ-5PW (20) 5 mL column
0045245	SkillPak TOYOPEARL NH2-750F 5 mL column
0045246	SkillPak TOYOPEARL GigaCap DEAE-650M 5 mL column
0045247	SkillPak TOYOPEARL GigaCap CM-650M 5 mL column
0045248	SkillPak TOYOPEARL QAE-550C 5 mL column
0045249	SkillPak TOYOPEARL SP-550C 5 mL column
Hydrophobic Interaction	Chromatography
0045250	SkillPak TOYOPEARL Butyl-600M 5 mL column
0045251	SkillPak TOYOPEARL Butyl-650M 5 mL column
0045252	SkillPak TOYOPEARL Phenyl-600M 5 mL column
0045253	SkillPak TOYOPEARL Phenyl-650M 5 mL column
0045254	SkillPak TOYOPEARL PPG-600M 5 mL column
0045255	SkillPak TOYOPEARL Hexyl-650C 5 mL column
0045256	SkillPak TOYOPEARL Ether-650M 5 mL column
Mixed Mode Chromatog	raphy
0045261	SkillPak TOYOPEARL MX-Trp-650M 5 mL column
Size Exclusion Chromato	ography
0045260	SkillPak TOYOPEARL HW-40F 5 mL column
HA Chromatography	
0045262	SkillPak Ca ⁺⁺ Pure-HA 5 mL column
Column Libraries	
0045263	SkillPak mAb Platform 5 mL column library, 5 mL × 1 each (TOYOPEARL AF-rProtein A HC-650F, Sulfate-650F, NH ₂ -750F)
0045264	SkillPak Salt Tolerant 5 mL column library, 5 mL \times 1 each (TOYOPEARL Sulfate-650F, NH2-750F)
0045265	SkillPak Mixed Mode 5 mL column library, 5 mL × 1 each (Ca ⁺⁺ Pure-HA, TOYOPEARL MX-Trp-650M)
0045266	SkillPak Best-in-Class 5 mL column library, 5 mL x 1 each (Ca ⁺⁺ Pure-HA, TOYOPEARL AF-rProtein A HC-650F, AF-rProtein L-650F, NH ₂ -750F, Sulfate-650F)

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