BioBasic® Columns
TG01-13

The best choice for biomolecules

• Analyze • Detect • Measure • Control™

Thermo
ELECTRON CORPORATION

Introduction

BioBasic columns are designed specifically for biochromatography of proteins, peptides, and nucleic acids. The 300Å pore size, high purity silica, and stable bonding chemistry of BioBasic packings makes them ideal for life science applications, including multidimensional LC/MS analyses. Available in a range of reversed phase, anion exchange, cation exchange and size exclusion columns, BioBasic columns meet all of your protein and peptide separation needs.

Applications

- Proteins and peptides
- Nucleotides
- Aromatic acids
- Sugars
- Food additives
- Antibiotics
- 2D Proteomics

Improved HPLC Performance for Biomolecules

Whether you're doing reversed phase or ion exchange, the BioBasic family of HPLC columns provides superior performance:

- improved resolution
- longer column lifetimes
- better reproducibility
- more efficient separations

Better Reproducibility

BioBasic columns provide superior chromatography, run after run, column after column. The extra dense bonding chemistry used for BioBasic reversed phase packings gives a highly stable,reproducible surface for reliable results. Figure 1 demonstrates the reproducible performance of 3 batches of BioBasic 18 packing materials.

Longer Column Lifetimes

BioBasic packings are designed to hold up to harsh mobile phase conditions for longer column lifetimes. Even when subjected to acid hydrolysis conditions at pH 1.8 and 50°C using 0.1% TFA, BioBasic columns show superior results. Figure 2 demonstrates a comparison of column lifetime under these difficult gradient conditions.

A Range of Stationary Phases with Different Selectivities

BioBasic reversed phase packings are available in several chemistries, including C18, C8, C4, phenyl and cyano. BioBasic ion exchangers include BioBasic AX for anion exchange and SCX for cation exchange. Table 1 provides a summary of characteristics for each BioBasic packing:

- 300Å pore size for improved reversed phase peptide and protein separations
- Outstanding reproducibility, stability and efficiency
- A range of stationary phases to optimize selectivity
- BioBasic AX and SCX silica-based ion exchange columns
- Capillary columns for multidimensional LC/MS analyses

Not Just for Large Molecules

BioBasic columns are designed to give superior results for the chromatography of proteins, peptides and biomolecules. However, BioBasic columns also give outstanding results for small molecule separations, whether by reversed phase or ion exchange. Please refer to the chromatograms that demonstrate the usefulness of BioBasic columns for proteins, peptides, and oligonucleotides, as well as a number of small molecule applications.

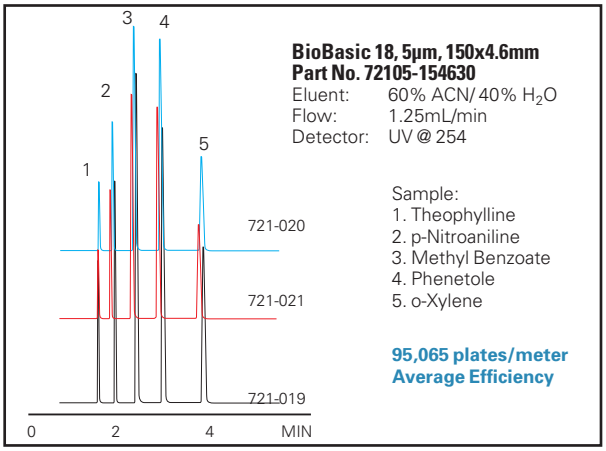


figure 1 - Nucleosides

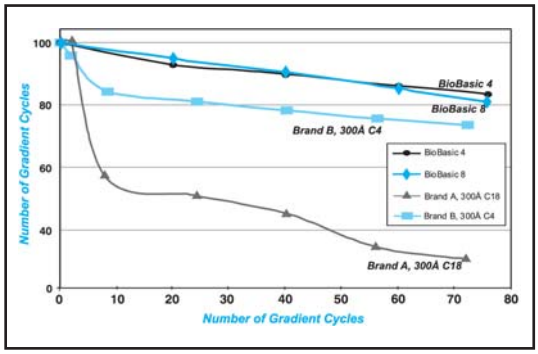
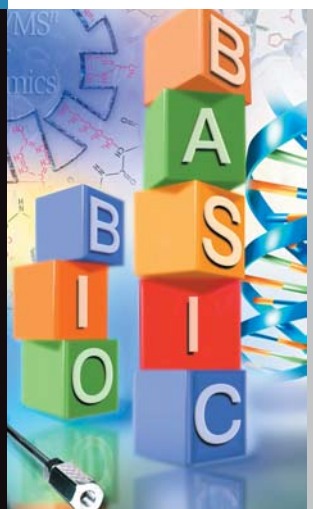


figure 2 - Acid Hydrolysis Study



Phase	Particle Size	Carbon Load	Pore Size	End-Capping	Silica Type
BioBasic 18	5µm	9%	300Å	yes	
BioBasic 8	5µm	5%	300Å	yes	
BioBasic 4	5µm	4%	300Å	yes	high purity base
BioBasic Phenyl	5µm	3%	300Å	yes	deactivated
BioBasic CN	5µm	3.5%	300Å	yes	
BioBasic AX	5µm	-	300Å	-	
BioBasic SCX	5µm	-	300Å	-	

table 1 - BioBasic Phase Characteristics

300Å Pore Size for Better Protein and Peptide Separations

Pore size can exert a significant influence on the chromatography of biomolecules. Figure 3 shows the higher resolution of the 300Å pore size BioBasic 18 column for a tryptic digest separation, compared to a 150Å pore size C18 column.In particular, the peaks eluting near 8 and 13 minutes show much higher resolution on the BioBasic 18 column. Figure 4 demonstrates the changes in selectivity that occur due to differences in bonded phase chain length. The BioBasic 18 and BioBasic 4 columns show a reversal of peak order for the two myoglobin peaks, which are not separated on the BioBasic 8 column. Figure 5 illustrates the separation of a mixture of five whole proteins on a BioBasic 18 KAPPA capillary column using a Finnigan™ LCQ Deca XP ion trap with components identified using BioWorks™ software.

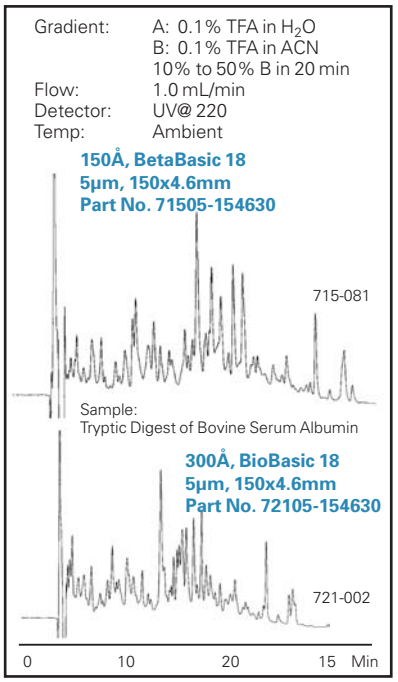


figure 3 - Effect of Pore Size on Tryptic Digest Separation

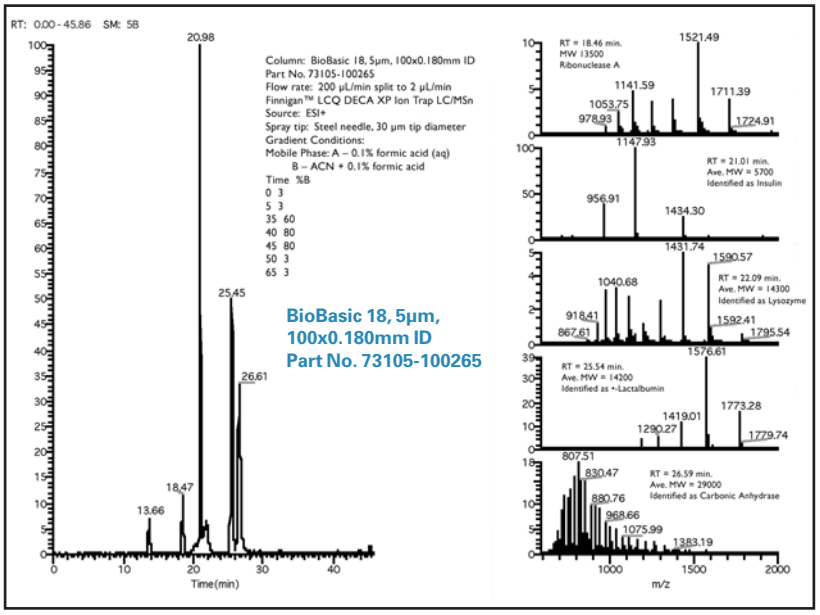


figure 5 - Effect of Pore Size on Protein Separation

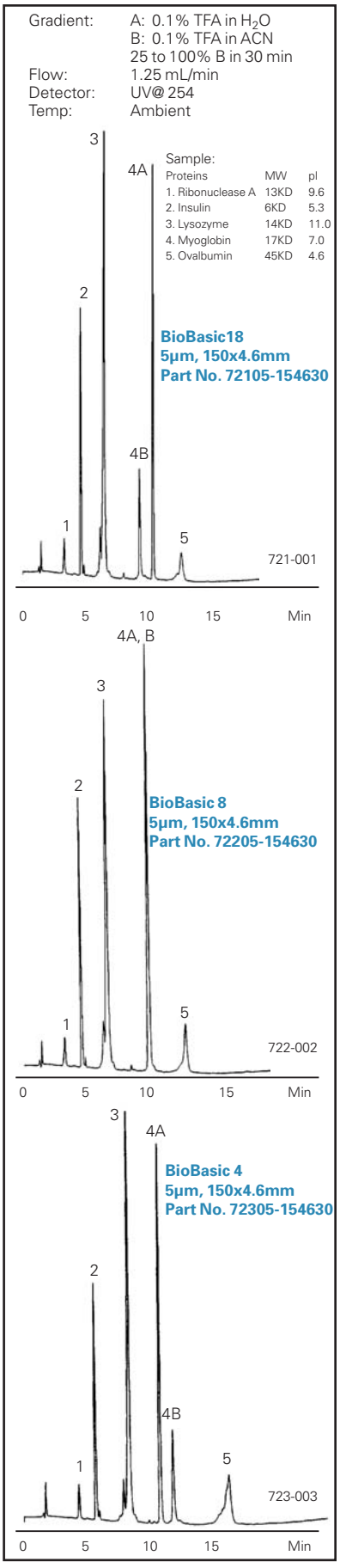


figure 4 - Effect of Bonded Phase Chain Length on Protein Separation

BioBasic AX Anion Exchange Columns
The BioBasic family now includes BioBasic AX columns for anion exchange. BioBasic AX columns give you superior performance for proteins, peptides and nucleic acids (figure 6), using protein-friendly ion exchange conditions.

BioBasic AX columns give you:
- better reproducibility
- longer column lifetimes
- more efficient separations
- multiple modes of interaction

BioBasic AX Packing Material
BioBasic AX columns are made from polyethyleneimine (PEI) covalently bonded to a highly base deactivated 300Å, 5µm silica (see table 2). PEI forms a polymeric structure on the silica surface which is protein-friendly. The covalent bond to the silica surface provides better stability and longer column lifetime than typical PEI-coated particles.

BioBasic AX columns can also be used in non-buffered conditions for Hydrophilic Interaction Liquid Chromatograpy (HILIC). HILIC is closely related to normal phase chromatography, although HILIC uses a high aqueous component in the mobile phase, as shown in figure 7.

Versatile BioBasic SCX Cation Exchange Columns
- Versatile cation exchanger
- 300Å pore size for better protein and peptide separations
- Superb stability under demanding pH conditions
- Exceptional efficiency from 5µm silica particles

BioBasic AX, 5µm, 150x4.6mm
Part No. 73105-154630
Gradient: A:5mM KH_2PO_4, pH 3.2
B:0.75M KH_2PO_4, pH 3.2
0-100%B in 30 min.
Flow: 1.0 mL/min
Detector: UV @ 254

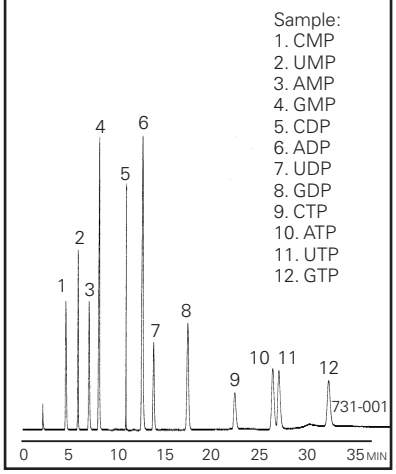


figure 6 - Nucleotides

BioBasic AX, 5µm, 150x4.6mm
Part No. 73105-154630
Gradient: A:95% ACN / 5% 5mM NH_4Formate, pH 6.13
B:80% ACN / 20% 5mM NH_4Formate, pH 6.13
0% B 0-5 min., 0-100%B 5-15 min.
Flow: 1.0 mL/min
Detector: ELSD

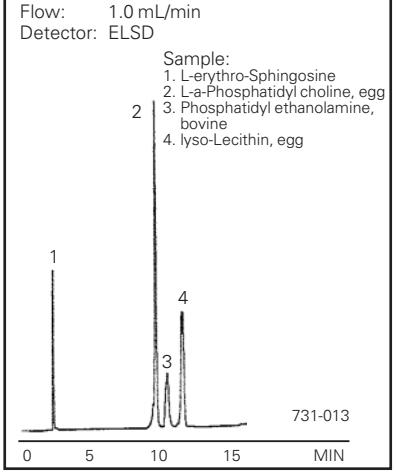


figure 7 - Phospholipids in HILIC Mode

Better Ion Exchange Performance
BioBasic SCX columns are designed to give superior reproducibility, both column-to-column and batch-to-batch. The 5µm, 300Å silica provides significantly higher efficiency than typical polymer-based ion exchangers. Every BioBasic SCX column packed and tested shows over 80,000 plates per meter as tested under normal phase conditions.

BioBasic SCX, 5µm, 150x4.6mm
Part No. 73205-154630
Gradient: A: 0.02M Tris, pH 6
B:A + 1.0M Na Acetate, pH 6
0-100%B in 60 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Sample: β-Lactoglobulin A tryptic digest

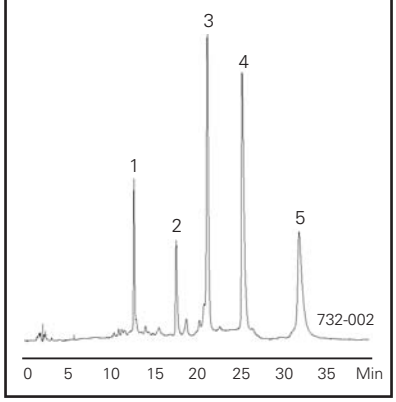
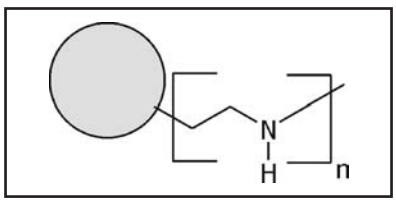


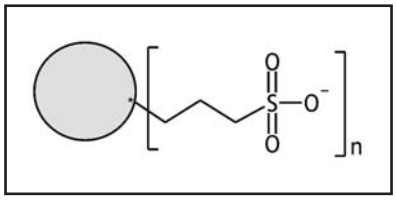
figure 8 - Proteins

Phase	Particle size	Pore size	Ion exchange ligand	Ion exchange capacity
BioBasic AX mequivalents/gram	5µm	300Å	PEI	0.22
BioBasic SCX mequivalents/gram	5µm	300Å	Sulfonic Acid	0.07

table 2 - BioBasic Ion Exchange Charateristics



Structure of PEI



Sulfonic Acid Structure

"Tunable" Ion Exchange

Both the BioBasic AX and BioBasic SCX packing materials can be used across a broad range of both pH and ionic strength. By manipulating buffer concentration, separations can be optimized for maximum retention, high efficiency, or rapid throughput.

Figures 9 and 10 demonstrate the effect of buffer concentration on retention for a series of organic acids using the BioBasic AX column. As buffer concentration decreases, retention increases. Figure 11 shows the same effect of buffer strength on the BioBasic SCX column for a series of basic analytes. In figure 12, the change in retention with change in pH is demonstrated on the BioBasic SCX column, showing longer retention and improved separations with decreased pH for a series of peptides.

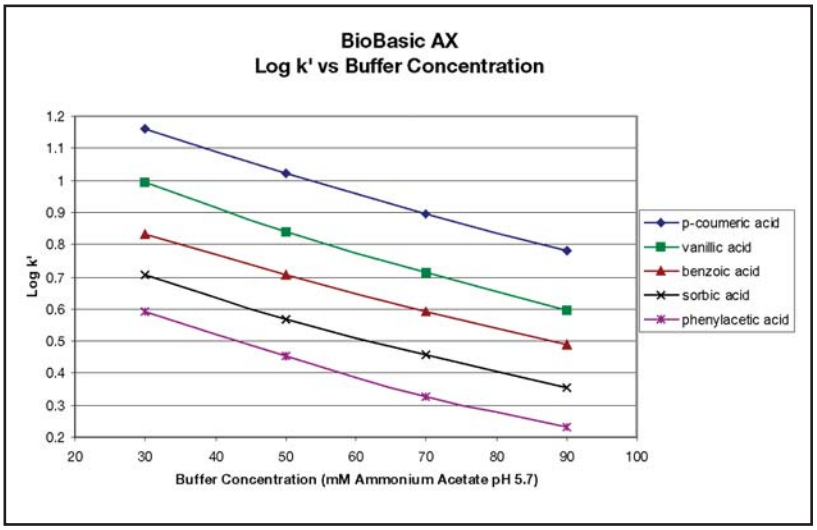


figure 9 - Effect of Buffer Concentration on Ion Exchange Retention

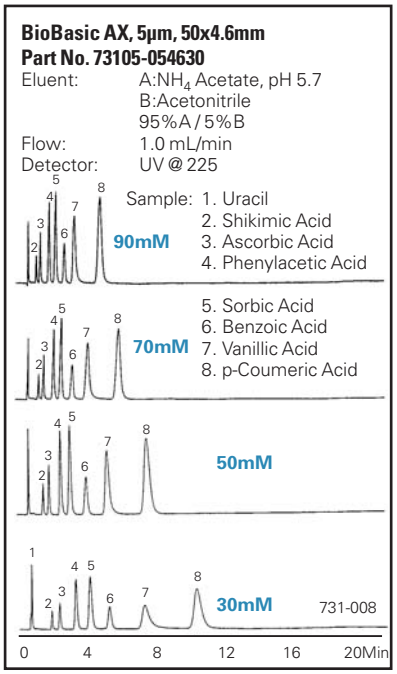


figure 10 - Effect of Buffer Concentration on BioBasic AX

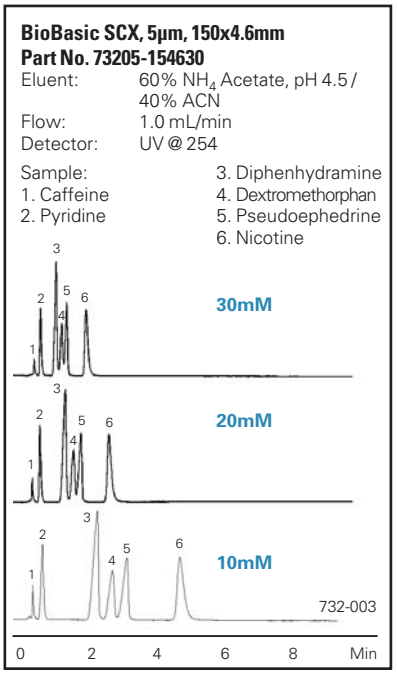


figure 11 - Effect of Buffer Concentration on BioBasic SCX

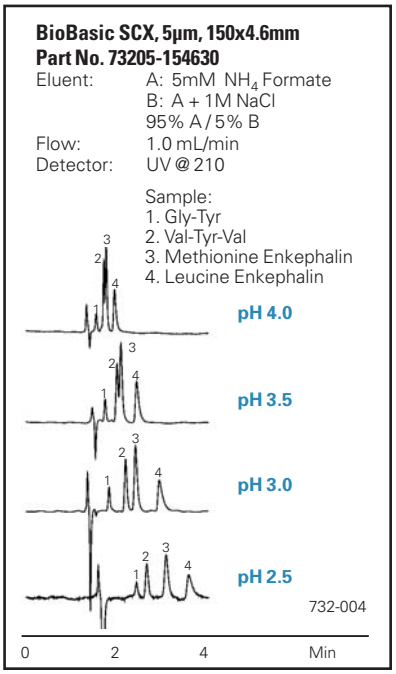


figure 12 - Effect of pH on BioBasic SCX

Better Performance from a Better Ion Exchange Column

BioBasic® AX columns are designed to give superior reproducibility, both column-to-column and batch-to-batch. Stringent quality control tests using ionic analytes and buffered mobile phase conditions ensure a reproducible ion exchange surface and consistent results.The exceptional ion exchange reproducibility of the BioBasic AX packing is demonstrated in figure 13.

BioBasic AX columns provide superb stability at both low and high pH ranges, unlike many other silica-based ion exchangers. Figures 14 and 15 show column lifetime studies done with 14,000 – 16,000 column volumes at pH 2.5 and pH 8.
At these pH extremes, many silica-based columns will show permanent degradation of the bonded phase (pH 2.5) or the underlying silica (pH 8). At either pH, the BioBasic AX columns are easily regenerated with a salt gradient to remove impurity buildup caused by the buffers, and return to their original performance. Ion exchange columns often show poor efficiency, especially those that are based on polymeric materials. The BioBasic AX packing is manufactured on a base-deactivated silica, which provides exceptional efficiency in an ion exchange column. In addition, the surface characteristics of the PEI-bonded BioBasic AX stationary phase provide rapid mass transfer for more efficient separations. Figure 16 shows a standard quality control test for the BioBasic AX packing run under normal phase conditions, demonstrating a minimum efficiency of 80,000 plates per meter for each column packed and tested

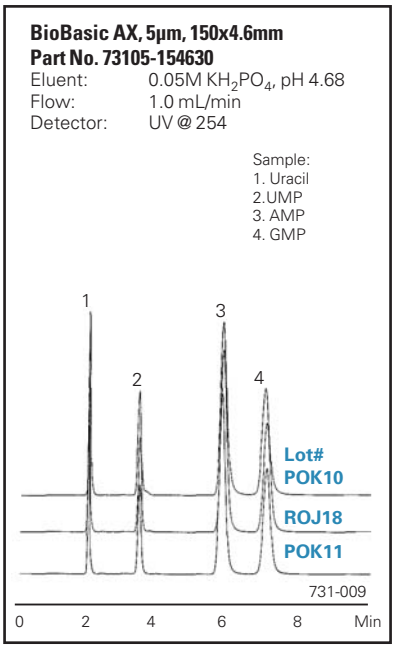


figure 13 - BioBasic AX Batch-to-Batch Reproducibility

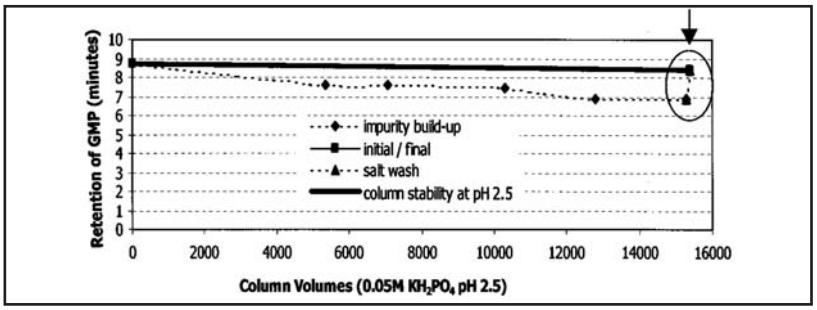


figure 14 - Stability of BioBasic AX at pH 2.5

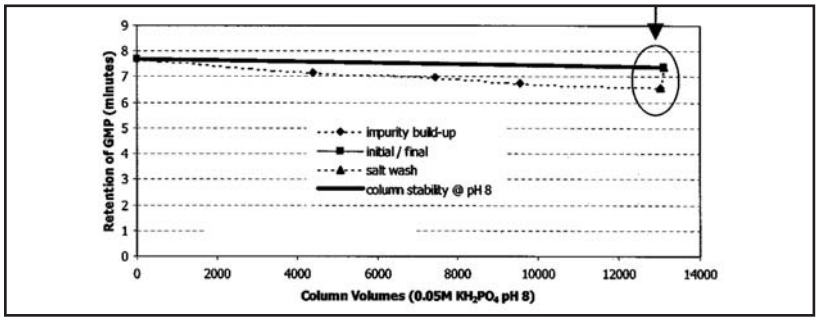


figure 15. - Stability of BioBasic AX at pH 8

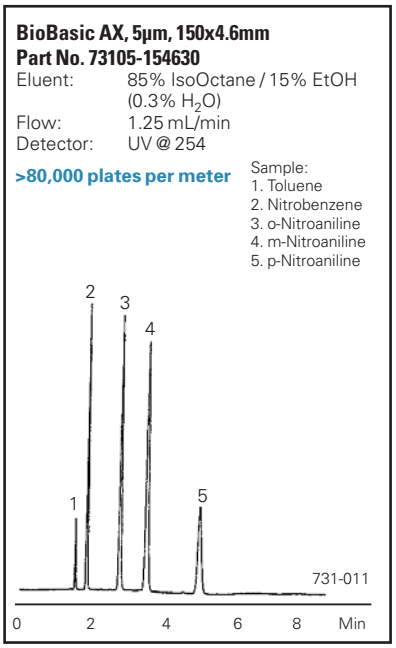
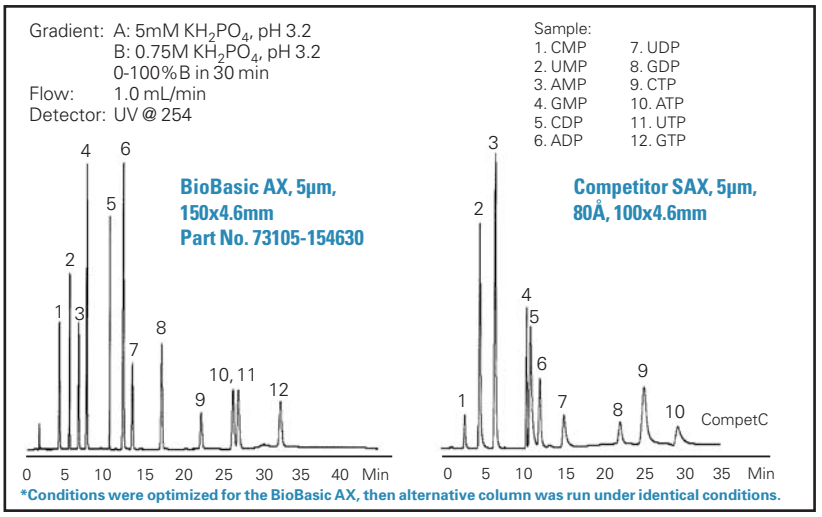


figure 16 - Individual Column Test

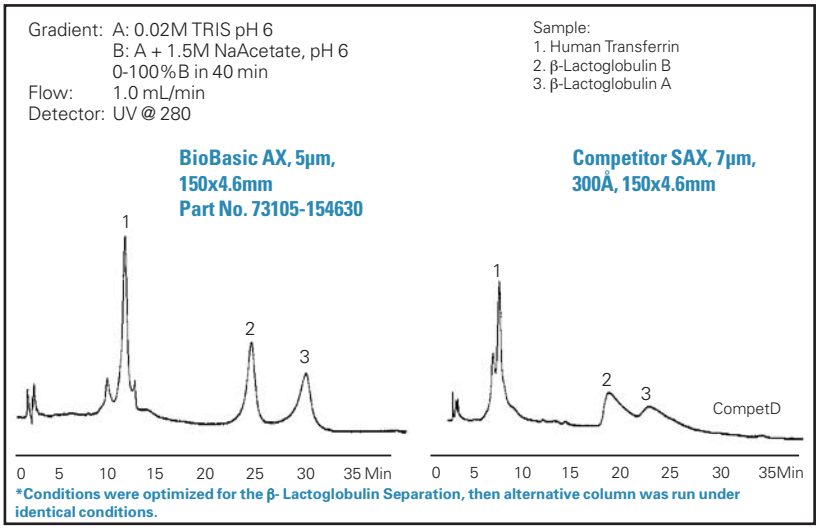
Better Performance from Better Biochromatography Columns

BioBasic AX columns provide better separations than many other choices for ion exchange of biological molecules. Figures 17 and 18 show comparisons of superior performance for both nucleic acids and proteins versus other choices for ion exchangers. Figure 17 demonstrates the excellent selectivity and resolution of the BioBasic AX column for a complex mixture of nucleotides compared to a popular 80Å pore size anion exchange column. Figure 18 shows three proteins chromatographed with good separation and efficiency on the BioBasic AX column versus a popular 300Å anion exchanger. BioBasic 18 columns also demonstrate superior performance when compared to other wide-pore reversed phase HPLC columns. Compare the difference in resolution for a BioBasic 4 column and another 300Å C4 column. Figure 19 demonstrates the higher resolving power of the BioBasic 4 column for a complex protein mixture, showing an additional peak separated with baseline resolution.



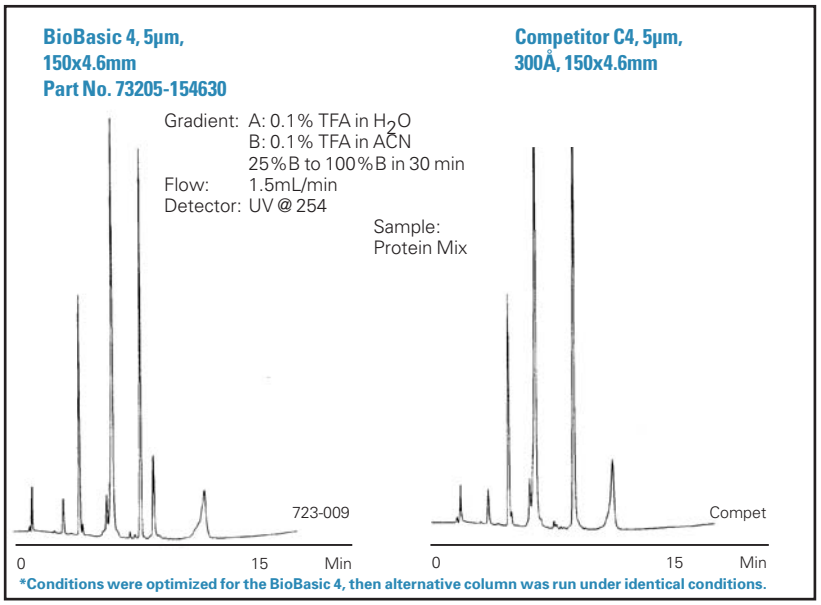
*Conditions were optimized for the BioBasic AX, then alternative column was run under identical conditions.

figure 17 - BioBasic AX Comparative Nucleotide Separation



*Conditions were optimized for the β- Lactoglobulin Separation, then alternative column was run under identical conditions.

figure 18 - BioBasic AX Comparative Nucleotide Separation



*Conditions were optimized for the BioBasic 4, then alternative column was run under identical conditions.

figure 19 - BioBasic® 18 Comparative Protein Separation

BioBasic® SCX for 2D Proteomics

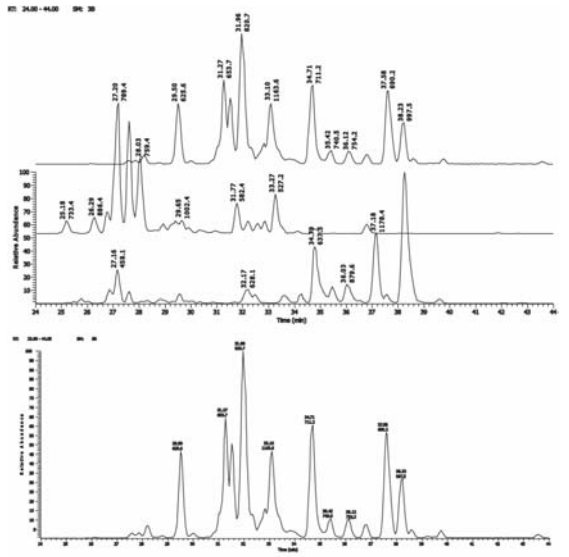
Proteomics is the study of proteins and their interaction within organisms, including the human body. Diseased states can frequently be traced to problems with protein expression and interaction. Proteomics accelerates drug discovery, since over 95 percent of all pharmaceuticals target proteins. Now that the genome has been sequenced, accelerated protein research is the next logical step, assisted by rapid improvements in the existing technologies.

Much of the work in proteomics is conducted with electrophoresis and 2-D gels. However, the volume of work required calls for methods and tools that offer greater speed and sensitivity to produce rapid breakthroughs. The Finnigan™ ProteomeX™ Workstation is one such tool, combining HPLC and tandem MS with ion trap technology. 2D proteomics combines reversed phase and ion exchange chromatography to increase the efficiency of protein identification methods (see figure 20). Co-eluting components can be separated into different fractions, in effect removing interferences from more abundant co-eluting species.
A typical two-dimensional LC/MS method involves separating fractions of protein digests by cation exchange chromatography. These separated fractions are then analyzed by reversed phase chromatography with MS/MS detection.

A BioBasic SCX capillary column is used for the initial ion exchange separation. The columns have been designed to elute analytes with relatively low ionic strength buffer (salt) gradients, particularly for analysis of small organic molecules, nucleotides, peptides and small proteins. In addition, the columns are LC/MS compatible because volatile buffers can be employed at low ionic strength, often with volatile organic modifiers present.
A BioBasic C18 capillary column is used for the reversed phase separation, based on a 300Å silica to ensure accuracy in quantification.

Sample Loading/Fraction Eluting

The sample of protein digest is loaded onto the 100x0.32mm ID BioBasic SCX cation exchange column using a mobile phase of 0.01% formic acid (0.1% for later fractions) at a flow rate of 2 µL/min (100:1 split flow) for a period of 20 minutes. To elute the fractions onto the 100x0.18mm ID BioBasic 18 reversed phase column, sequential 20 µL injections of increasing concentrations of NH_4CO_2H (0mM, 20mM, 50mM, 100mM, 200mM, in 0.1% formic acid) were used. Three of these fractions are shown in figure 20.



Sample: BSA Digest

Fractionation from BioBasic SCX
Part No. 73205-100365

Column Dimensions:
Inj. volume: 3µL (1500 fmol total digest on column)

Fractions eluted with 20µL Ammonium Formate (concentrations listed, in 0.1% Formic Acid) onto 100x0.180mm ID BioBasic 18 capillary

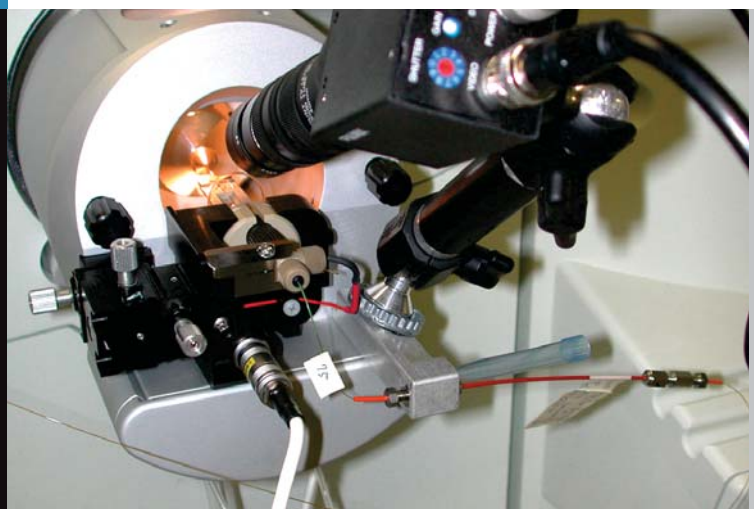
Reversed Phase Separation of BSA Digest
Part No. 72105-100265

Column Dimensions: 100x0.32mm ID
Mobile Phase: A: 0.01% Formic Acid
B: ACN + 0.1% Formic Acid
Flow: 2 µL/min
Gradient:

Time	%B
0	0
3	0
65	60

Ionization Method: ESI+
Sheath Gas: 10 arb units
Capillary Temp.: 130°C
Spray Voltage: 2.5 kV
Capillary Voltage: 12.00 kV
Scan Range: 300-2000u

figure 20 - 2-Dimensional Protein Fractionation using BioBasic SCX and BioBasic 18 Capillary Columns

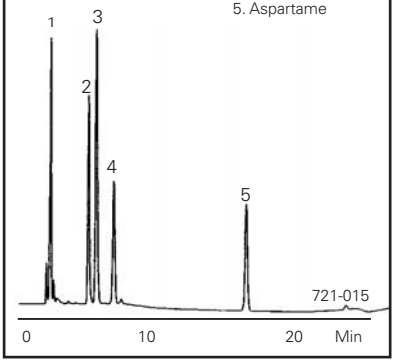


Food Additives

BioBasic 18, 5µm, 150x4.6mm
Part No. 72105-154630

Gradient: A: 0.05M KH_2PO_4, pH 6
B: MeOH
5% B to 50% B in 20 min.
Flow: 1 mL/min
Detector: UV@ 200

Sample:
1. Uracil
2. Acesulfame
3. Benzoic Acid
4. Sorbic Acid
5. Aspartame

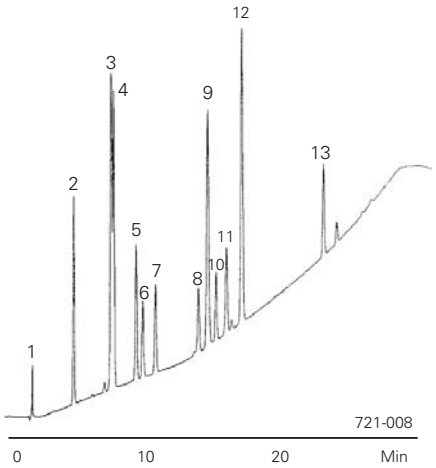


Aromatic Acids on BioBasic 18

BioBasic 18, 5µm, 150x4.6mm
Part No. 72105-154630

Eluent: A: 0.5M KH_2PO_4, pH 3
B: MeOH
10%B to 60%B in 30 min.
Flow: 1.0 mL/min
Detector: UV @ 200

Sample:
1. Uracil
2. Protocatachuic Acid
3. Caffeic Acid
4. p-Hydroxybenzoic Acid
5. Phthalic Acid
6. Vanillic Acid
7. Syringic Acid
8. Phenylacetic Acid
9. Benzoic Acid
10. Salicylic Acid
11. p-Coumeric Acid
12. Ferulic Acid
13. trans-Cinnamic Acid

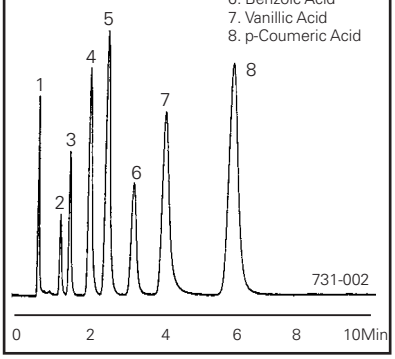


Aromatic Acids on BioBasic AX

BioBasic AX, 5µm, 50x4.6mm
Part No. 73105-054630

Eluent: A: 70mM NH_4Acetate, pH 5.7
B: Acetonitrile
95%A / 5%B
Flow: 1.0 mL/min
Detector: UV @ 225

Sample:
1. Uracil
2. Shikimic Acid
3. Ascorbic Acid
4. Phenylacetic Acid
5. Sorbic Acid
6. Benzoic Acid
7. Vanillic Acid
8. p-Coumeric Acid



Small Organic Acids - Variation with pH

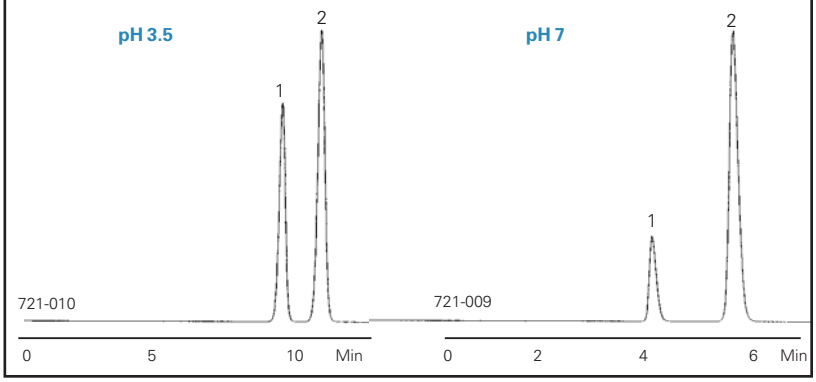
BioBasic® 18, 5µm, 150x4.6mm
Part No. 72105-154630

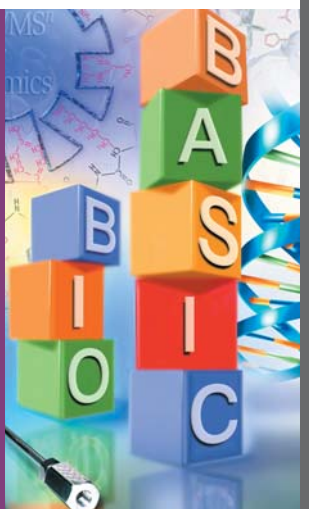
Eluent: A: 80% 0.05M KH_2PO_4, pH 3.5
B: 20% MeOH
Flow: 1.0 mL/min
Detector: UV @ 235

BioBasic 18, 5µm, 150x4.6mm
Part No. 72105-154630

Eluent: A: 90% 0.05M KH_2PO_4 in H_2O, pH 7
B: 10% MeOH
Flow: 1.0 mL/min
Detector: UV @ 235

Sample:
1. Benzoic Acid
2. Sorbic Acid



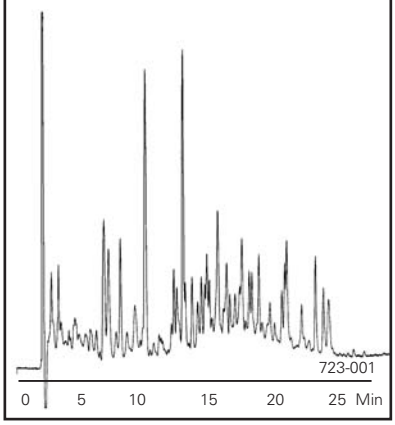


Tryptic Digest of BSA

BioBasic 4, 5µm, 150x4.6mm
Part No. 72305-154630

Gradient: A: 0.1% TFA in H_2O
B: 0.1% TFA in ACN
5% B - 50% B in 40 min.
Flow: 1.0 mL/min
Detector: UV @ 220

Sample: Tryptic digest of bovine serum albumin



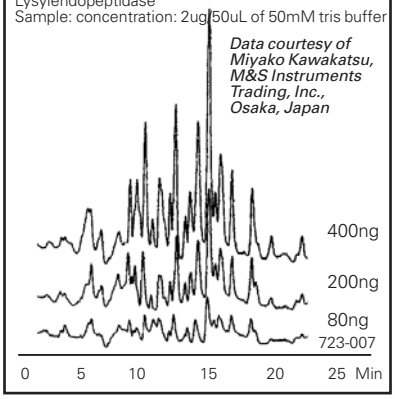
Peptide Fragments

BioBasic 4, 5µm, 150x1mm
Part No. 72305-151030

Gradient: A:0.1% TFA in H_2O
B: 0.1% TFA in 59%ACN
5-100% to B in 30 min.
Hold for 5 min.
Flow: 60 µL/min
Detector: UV@ 210
System: Gilson small bore HPLC

Sample: Peptide fragments from BSA treated with Lysylendopeptidase
Sample: concentration: 2ug/50uL of 50mM tris buffer

Data courtesy of Miyako Kawakatsu, M&S Instruments Trading, Inc., Osaka, Japan

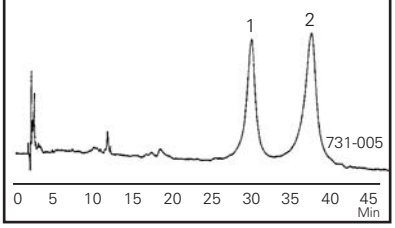


β-Lactoglobulins

BioBasic AX, 5µm, 150x4.6mm
Part No. 73105-154630

Gradient: A: 0.02M TRIS, pH 6
B: A + 1M NaAcetate, pH 6
0-100%B in 40 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Sample: 1. β-Lactoglobulin B
2. β-Lactoglobulin A

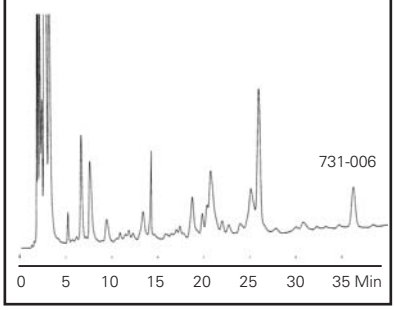


Tryptic Digest of b-Lactoglobulin A

BioBasic AX, 5µm, 150x4.6mm
Part No. 73105-154630

Gradient: A:0.02M TRIS, pH 6
B:A + 1M NaAcetate, pH 6
0-100%B in 50 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Sample: β-Lactoglobulin A tryptic digest

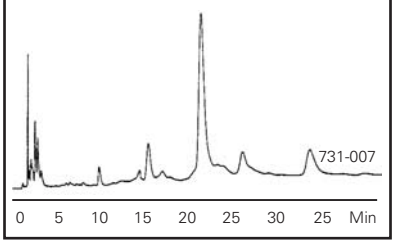


Tryptic Digest of Ovalbumin

BioBasic AX, 5µm, 150x4.6mm
Part No. 73105-154630

Gradient: A: 0.02M TRIS, pH 6
B: A + 1M NaAcetate, pH 6
0-100%B in 50 min.
Flow: 1.0 mL/min
Detector: UV @ 280

Sample: Tryptic Digest of Ovalbumin

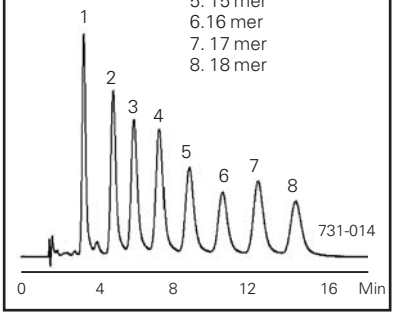


Oligonucleotides

BioBasic AX, 5µm, 50x4.6mm
Part No. 73105-054630

Gradient: A: 5mM KH_2PO_4, pH 7.2
B: 0.15M KH_2PO_4, pH 7.2
75-100%B in 15 min.
Flow: 0.4 mL/min
Detector: UV @ 265

Sample:
Oligothymidylic Acid
1. 10 mer
2. 12 mer
3. 13 mer
4. 14 mer
5. 15 mer
6. 16 mer
7. 17 mer
8. 18 mer



Standard HPLC Columns

Description	Particle Size(µm)	Length (mm)	Standard bore (4.6 mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0 mm)
BioBasic 18	5	50	72105-054630	72105-054030	72105-053030	72105-052130	72105-051030
	5	100	72105-104630	72105-104030	72105-103030	72105-102130	72105-101030
	5	150	72105-154630	72105-154030	72105-153030	72105-152130	72105-151030
	5	250	72105-254630	72105-254030	72105-253030	72105-252130	72105-251030
BioBasic 8	5	50	72205-054630	72205-054030	72205-053030	72205-052130	72205-051030
	5	100	72205-104630	72205-104030	72205-103030	72205-102130	72205-101030
	5	150	72205-154630	72205-154030	72205-153030	72205-152130	72205-151030
	5	250	72205-254630	72205-254030	72205-253030	72205-252130	72205-251030
BioBasic 4	5	50	72305-054630	72305-054030	72305-053030	72305-052130	72305-051030
	5	100	72305-104630	72305-104030	72305-103030	72305-102130	72305-101030
	5	150	72305-154630	72305-154030	72305-153030	72305-152130	72305-151030
	5	250	72305-254630	72305-254030	72305-253030	72305-252130	72305-251030
BioBasic CN	5	50	72905-054630	72905-054030	72905-053030	72905-052130	72905-051030
	5	100	72905-104630	72905-104030	72905-103030	72905-102130	72905-101030
	5	150	72905-154630	72905-154030	72905-153030	72905-152130	72905-151030
	5	250	72905-254630	72905-254030	72905-253030	72905-252130	72905-251030
BioBasic Phenyl	5	50	72405-054630	72405-054030	72405-053030	72405-052130	72405-051030
	5	100	72405-104630	72405-104030	72405-103030	72405-102130	72405-101030
	5	150	72405-154630	72405-154030	72405-153030	72405-152130	72405-151030
	5	250	72405-254630	72405-254030	72405-253030	72405-252130	72405-251030
BioBasic AX	5	50	73105-054630	73105-054030	73105-053030	73105-052130	73105-051030
	5	100	73105-104630	73105-104030	73105-103030	73105-102130	73105-101030
	5	150	73105-154630	73105-154030	73105-153030	73105-152130	73105-151030
	5	250	73105-254630	73105-254030	73105-253030	73105-252130	73105-251030
BioBasic SCX	5	50	73205-054630	73205-054030	73205-053030	73205-052130	73205-051030
	5	100	73205-104630	73205-104030	73205-103030	73205-102130	73205-101030
	5	150	73205-154630	73205-154030	73205-153030	73205-152130	73205-151030
	5	250	73205-254630	73205-254030	73205-253030	73205-252130	73205-251030

Drop-In Guard Cartridges (pk/4)

NOTE: 4.0mm drop-ins are used for both 4.0 and 4.6mm analytical columns.

Description	Particle Size(µm)	Length (mm)	Standard bore (for 4.6 mm)	Standard bore (4.0 mm)	Small bore (3.0 mm)	Small bore (2.1 mm)	Microbore (1.0 mm)
BioBasic 18	5	10	72105-014001	72105-014001	72105-013001	72105-012101	72105-011001
BioBasic 8	5	10	72205-014001	72205-014001	72205-013001	72205-012101	72205-011001
BioBasic 4	5	10	72305-014001	72305-014001	72305-013001	72305-012101	72305-011001
BioBasic CN	5	10	72905-014001	72905-014001	72905-013001	72905-012101	72905-011001
BioBasic Phenyl	5	10	72405-014001	72405-014001	72405-013001	72405-012101	72405-011001
BioBasic AX	5	10	73105-014001	73105-014001	73105-013001	73105-012101	73105-011001
BioBasic SCX	5	10	73205-014001	73205-014001	73205-013001	73205-012101	73205-011001
UNIGUARD Direct-Connection Drop-In Guard Cartridge Holder for use with standard columns 		10	850-00	850-00	852-00	852-00	851-00

KAPPA Capillary Columns

Description	Particle Size(µm)	Length (mm)	ID 500µm	ID 320µm	ID 180µm	ID 100µm	ID 75µm
BioBasic 18	5	100	72105-100565	72105-100365	72105-100265	72105-100165	72105-100065
	5	150	72105-150565	72105-150365	72105-150265	72105-150165	72105-150065
	5	250	72105-250565	72105-250365	72105-250265	72105-250165	72105-250065
BioBasic 8	5	100	72205-100565	72205-100365	72205-100265	72205-100165	72205-100065
	5	150	72205-150565	72205-150365	72205-150265	72205-150165	72205-150065
	5	250	72205-250565	72205-250365	72205-250265	72205-250165	72205-250065
BioBasic SCX	5	100	73205-100565	73205-100365	73205-100265	73205-100165	73205-100065
	5	150	73205-150565	73205-150365	73205-150265	73205-150165	73205-150065
	5	250	73205-250565	73205-250365	73205-250265	73205-250165	73205-250065

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