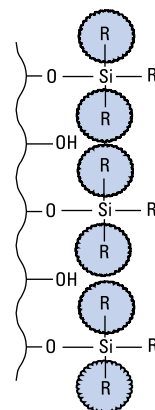


## ZORBAX 300 Å StableBond

Agilent ZORBAX 300 Å StableBond columns are an ideal choice for the reproducible separations of proteins and peptides for two key reasons. First, wide-pore, 300 Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, to allow these analytes to completely access the bonded phase. Second, 300StableBond columns are unmatched in their durability at low pH, such as with TFA-containing mobile phases typically used for protein and peptide separations. For LC/MS separations at low pH, 300StableBond columns can also be used with formic acid and acetic acid mobile phase modifiers. These columns are available in five different bonded phases (C18, C8, C3, CN, and diphenyl (DP)) for selectivity and recovery optimization of proteins and polypeptides. To further increase sample recovery and improve efficiency for difficult proteins, 300StableBond columns can be used up to 80 °C. StableBond 300SB-C18 and 300SB-C8 columns are an ideal choice for complex protein and protein digest separations. These columns are also available in capillary (0.3 and 0.5 mm id) and nano (0.075 and 0.10 mm id) dimensions for reversed-phase LC/MS separations of protein digests. Capillary and nano columns can be used for either 1D or 2D proteomics separations.



Sterically Protected 300StableBond bonded phase

### UHPLC Column Specifications

Bonded Phase	Pore Size	Temp Limits*	pH Range*	Endcapped
ZORBAX RRHD 300SB-C18	300 Å	90 °C	1.0-8.0	No
ZORBAX RRHD 300SB-C8	300 Å	80 °C	1.0-8.0	No
ZORBAX RRHD 300SB-C3	300 Å	80 °C	1.0-8.0	No
ZORBAX RRHD 300-Diphenyl	300 Å	80 °C	1.0-8.0	Yes
ZORBAX 300SB-C18	300 Å	80 °C	1.0-8.0	No
ZORBAX 300SB-C8	300 Å	80 °C	1.0-8.0	No
ZORBAX 300SB-C3	300 Å	80 °C	1.0-8.0	No
ZORBAX 300SB-CN	300 Å	80 °C	1.0-8.0	No

Specifications represent typical values only.

\* 300StableBond columns are designed for optimal use at low pH. At pH 6-8, the highest column stability for all silica-based columns is obtained by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01-0.02 M. At mid or high pH, 300Extend-C18 is recommended.

## Higher resolution of intact monoclonal antibody

**Column:** ZORBAX RRHD 300SB-C8  
857750-906  
2.1 x 50 mm, 1.8  $\mu$ m

**Mobile phase:** A: H<sub>2</sub>O:IPA (98:2) + 0.1% TFA (v/v)  
B: IPA:ACN:H<sub>2</sub>O (70:20:10) + 0.1% TFA (v/v)

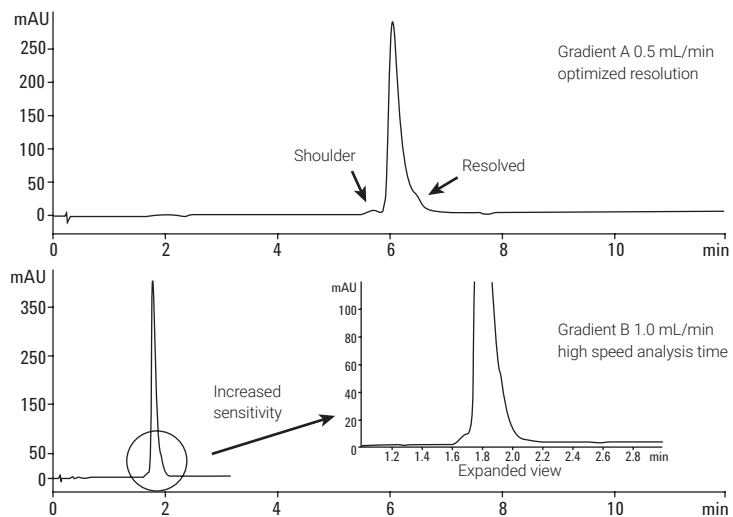
**Flow rate:** Between 0.5 mL/min and 1.0 mL/min

**Gradient:** Multisegmented and linear elution

**Temperature:** 80 °C

**Detector:** 1290 Infinity LC with autosampler, binary pump and thermostatted column compartment, and diode array detector (DAD)

**Sample:** UV, 225 nm



## Tips and tools

For more information on better characterization of biomolecules using AdvanceBio Reversed-Phase columns, see the white paper on this topic (publication 5991-2032EN).

[www.agilent.com/search](http://www.agilent.com/search)



## Reduced and alkylated mAb—separation of light chain and heavy chain variants

**Column:** ZORBAX RRHD 300SB-C8  
858750-906  
2.1 x 150 mm, 1.8  $\mu$ m

**Mobile phase:** A: H<sub>2</sub>O + 0.1% TFA (v/v)  
B: n-propanol:ACN:H<sub>2</sub>O (80:10:10) + 0.1% TFA (v/v)

**Flow rate:** 0.5 mL/min

**Injection:** 3  $\mu$ L (from 2.5 mg/mL sample)

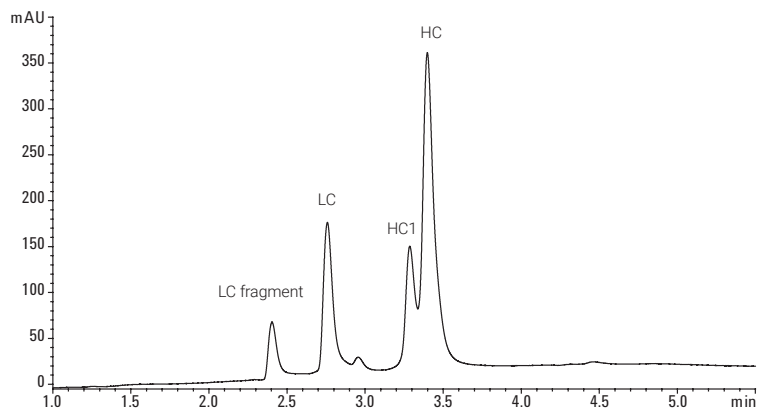
% Solvent B	Time (min)
20	0
35	3
40	4
40	5
90	5.1
90	5.5
25	6

**Gradient:** Multisegmented

**Temperature:** 75 °C

**Detector:** UV, 225 nm

**Instrument:** 1290 Infinity LC with autosampler, binary pump, thermostatted column compartment, and diode array detector (DAD)



For consecutive chromatographic runs, a 2-min post-run was added to re-equilibrate the column.

## Tips and tools

Typical mobile phases for protein and peptide separations combine a very low pH with TFA (or other acids) to solubilize proteins. StableBond columns have extremely long lifetimes under these conditions. They are available in 300 Å pore size for proteins up to 100–500 kDa.

### Improved reproducibility of monoclonal antibodies

**Column:** ZORBAX RRHD 300SB-C8  
857750-906  
2.1 x 50 mm, 1.8 µm

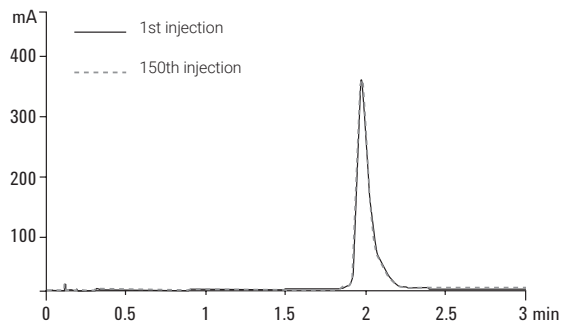
**Mobile phase:** A: H<sub>2</sub>O:IPA (98:2) + 0.1% TFA (v/v)  
B: IPA:ACN:H<sub>2</sub>O (70:20:10) + 0.1% TFA

**Flow rate:** 1.0 mL/min

**Temperature:** 80 °C

**Detector:** 1290 Infinity LC with diode array detector at 225 nm

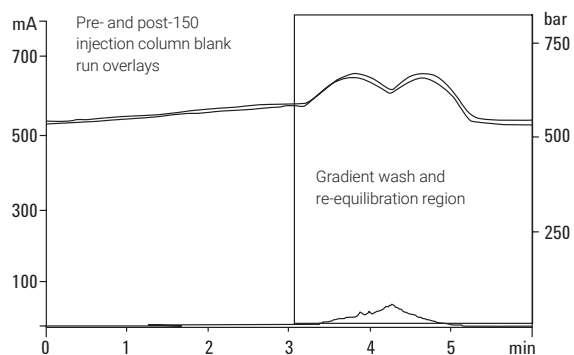
**Sample:** mAb



### Gradient

Particle size, µm	Flow rate, mL/min
0.00	25
3.00	35
4.00	90
5.00	25

Excellent column reproducibility and protein recovery using ZORBAX 300SB-C8.



## Unique selectivity choices for mAb characterization

**Columns:** ZORBAX RRHD 300SB-C18  
858750-902  
2.1 x 100 mm, 1.8  $\mu$ m

ZORBAX RRHD 300SB-C3  
858750-909  
2.1 x 100 mm, 1.8  $\mu$ m

ZORBAX RRHD 300SB-C8  
858750-906  
2.1 x 100 mm, 1.8  $\mu$ m

ZORBAX RRHD 300-Diphenyl  
858750-944  
2.1 x 100 mm, 1.8  $\mu$ m

Mobile phase: A: H<sub>2</sub>O (0.1% TFA) (v/v)  
B: 80% nPA:10% ACN:10% H<sub>2</sub>O (0.08% TFA) (v/v)

Injection volume: 3  $\mu$ L (from 2.5 mg/mL sample)

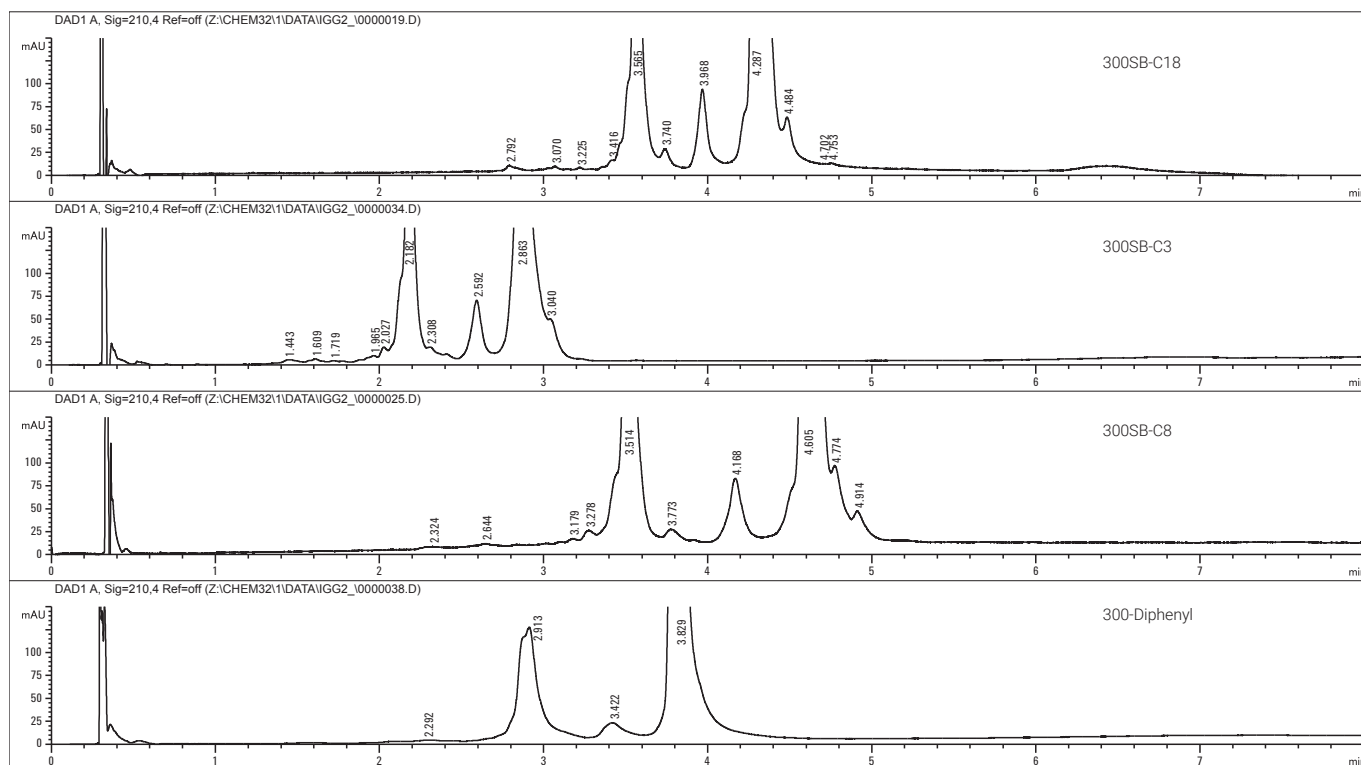
Flow rate: 1.0 mL/min (3.5  $\mu$ m\*), 1.0 mL/min (1.8  $\mu$ m)

Gradient: 25-35% B, 90% wash

Temperature: 80 °C

Detector: UV, 215 nm

\* Broad peaks at lower flow rates



Peptides/proteins: effect of elevated temperature

**Column:** ZORBAX RRHD 300SB-C3  
883995-909  
4.6 x 150 mm, 5 µm

**Mobile phase:** A: 5:95 ACN:water with 0.10% TFA (v/v%)  
B: 95:5 ACN:water with 0.085% TFA (v/v%)

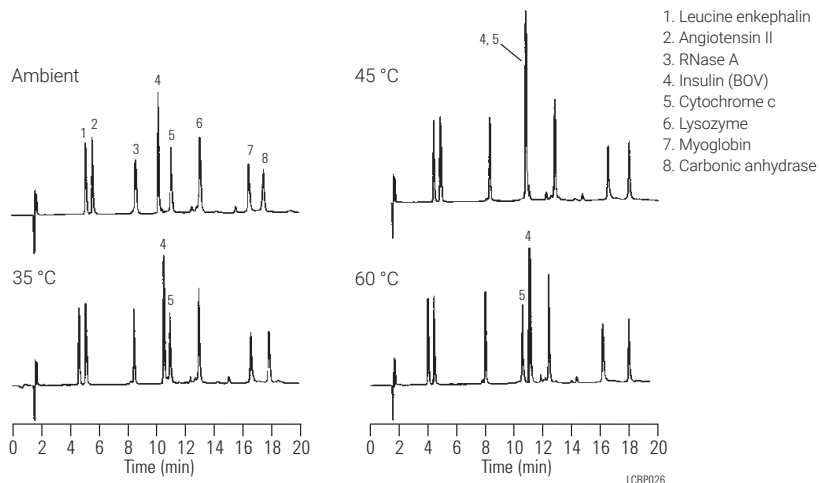
**Flow rate:** 1.0 mL/min

**Gradient:** 15-53% in 20 min, posttime 12 min

**Temperature:** Ambient-60 °C

**Detector:** UV, 215 nm

**Sample:** Polypeptides



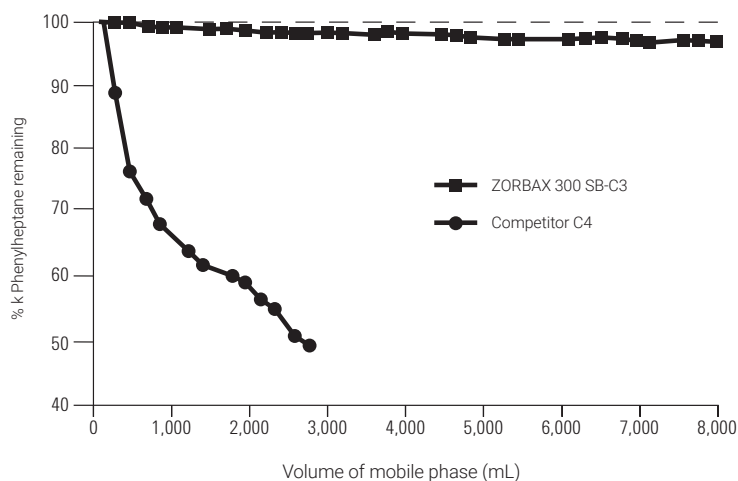
Short-chain ZORBAX 300SB-C3 is stable at low pH, high temperature

**Column:** ZORBAX 300SB-C3  
883995-909  
4.6 x 150 mm, 5 µm

**Mobile phase:** Gradient 0-100% B in 80 min  
A: 0.5% TFA in water  
B: 0.5% TFA in acetonitrile  
Isocratic retention test conditions:  
1-phenylheptane 50% A, 50% B

**Flow rate:** 1.0 mL/min

**Temperature:** 60 °C



## Four different 300SB bonded phases optimize separation of large polypeptides

**Column A:** ZORBAX RRHD 300SB-C18  
883995-902  
4.6 x 150 mm, 5 μm

**Column B:** ZORBAX 300SB-C8  
883995-906  
4.6 x 150 mm, 5 μm

**Column C:** ZORBAX 300SB-C3  
858750-909  
4.6 x 150 mm, 5 μm

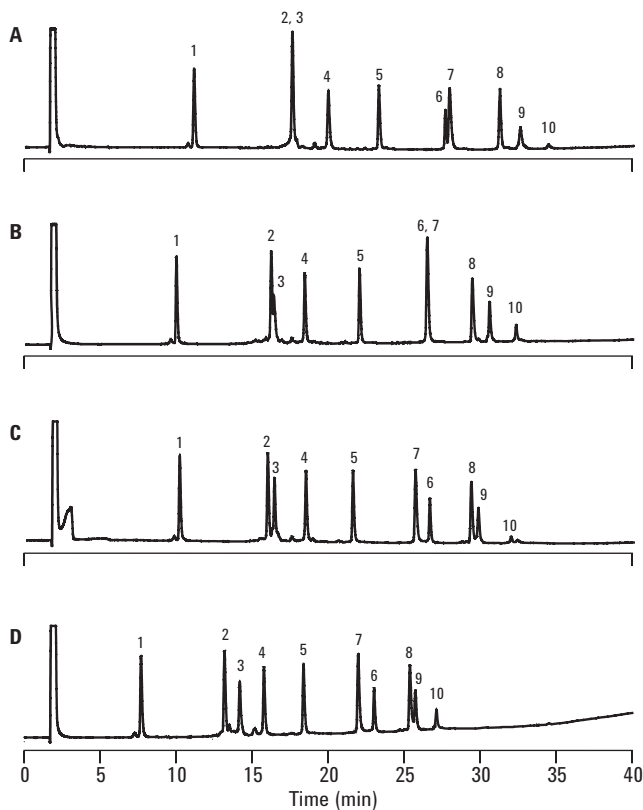
**Column D:** ZORBAX 300SB-CN  
858750-905  
4.6 x 150 mm, 5 μm

**Mobile phase:** Linear gradient, 25–70% B in 40 min  
A: 0.1% TFA in water  
B: 0.09% TFA in 80% acetonitrile:20% water

**Flow rate:** 1.0 mL/min

**Temperature:** 60 °C

**Sample:** 3 μg each protein



1. RNase
2. Insulin
3. Cytochrome c
4. Lysozyme
5. Parvalbumin
6. CDR
7. Myoglobin
8. Carbonic anhydrase
9. S-100 $\beta$
10. S-100 $\alpha$

LCSB006

The 300SB-C18, C8, C3, and CN bonded phases all provide a different separation of this group of polypeptides. This adds an important parameter for quickly optimizing protein separations. The 300SB-CN column offers unique selectivity for more hydrophilic polypeptides.

## ZORBAX 300 Å StableBond

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
<b>Standard columns (no special hardware required)</b>							
Semipreparative	9.4 x 250	5	880995-202	880995-206	880995-205	880995-209	
Analytical	4.6 x 250	5	880995-902	880995-906	880995-905	880995-909	
Analytical	4.6 x 150	5	883995-902	883995-906	883995-905	883995-909	
Analytical	4.6 x 50	5	860950-902	860950-906	860950-905	860950-909	
Rapid Resolution	4.6 x 150	3.5	863973-902	863973-906	863973-905	863973-909	
Rapid Resolution	4.6 x 100	3.5	861973-902	861973-906			
Rapid Resolution	4.6 x 50	3.5	865973-902	865973-906	865973-905	865973-909	
Solvent Saver Plus	3.0 x 150	3.5	863974-302	863974-306		863974-309	
Solvent Saver Plus	3.0 x 100	3.5		861973-306			
Narrow Bore	2.1 x 250	5	881750-902				
Narrow Bore	2.1 x 150	5	883750-902	883750-906	883750-905	883750-909	
Narrow Bore RR	2.1 x 150	3.5		863750-906			
Narrow Bore RR	2.1 x 100	3.5	861775-902	861775-906			
Narrow Bore RR	2.1 x 50	3.5	865750-902	865750-906			
Narrow Bore RRHD	2.1 x 100	1.8	858750-902	858750-906		858750-909	858750-944
Narrow Bore RRHD	2.1 x 50	1.8	857750-902	857750-906		857750-909	857750-944
MicroBore	1.0 x 250	5	861630-902				
MicroBore RR	1.0 x 150	3.5	863630-902	863630-906			
MicroBore RR	1.0 x 50	3.5	865630-902	865630-906			
MicroBore guard, 3/pk	1.0 x 17	5	5185-5920	5185-5920			
Guard cartridge, 2/pk	9.4 x 15	7	820675-124	820675-124	820675-124	820675-124	
Guard cartridge, 4/pk	4.6 x 12.5	5	820950-921	820950-918	820950-923	820950-924	

(Continued)

AB

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Part of the AdvanceBio family

## Tips and tools

To get the most out of your Agilent instruments, visit: [www.agilent.com/chem/getbioguides](http://www.agilent.com/chem/getbioguides)



# Primary Structure Analysis

## ZORBAX 300 Å StableBond

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
Guard cartridge, 4/pk	2.1 x 12.5	5	821125-918	821125-918	821125-924	821125-924	
Guard hardware kit			840140-901	840140-901	840140-901	840140-901	
Guard hardware kit			820999-901	820999-901	820999-901	820999-901	
<b>PrepHT cartridge columns (require endfittings kit 820400-901)</b>							
PrepHT cartridge	21.2 x 250	7		897250-102	897250-106	897250-105	897250-109
PrepHT cartridge	21.2 x 150	7		897150-102	897150-106		897150-109
PrepHT cartridge	21.2 x 150	5		895150-902	895150-906		895150-909
PrepHT cartridge	21.2 x 100	5		895100-902	895100-906		895100-909
PrepHT cartridge	21.2 x 50	5		895050-902	895050-906		895050-909
PrepHT endfittings, 2/pk				820400-901	820400-901	820400-901	820400-901
PrepHT guard	17.0 x 7.5	5		820212-921	820212-918	820212-924	820212-924
Guard cartridge hardware				820444-901	820444-901	820444-901	820444-901
<b>Capillary glass-lined columns</b>							
Capillary	0.5 x 250	5	5064-8266				
Capillary	0.5 x 150	5	5064-8264				
Capillary	0.5 x 35	5	5064-8294				
Capillary RR	0.5 x 150	3.5	5064-8268				
Capillary RR	0.5 x 35	3.5	5065-4459				
Capillary	0.3 x 250	5	5064-8265				
Capillary	0.3 x 150	5	5064-8263				
Capillary	0.3 x 35	5	5064-8295				
Capillary RR	0.3 x 150	3.5	5064-8267	5065-4460			
Capillary RR	0.3 x 100	3.5	5064-8259	5065-4461			
Capillary RR	0.3 x 35	3.5	5064-8270	5065-4462			
Capillary RR	0.3 x 50	3.5	5064-8300	5065-4463			
<b>Nano columns (PEEK fused silica)</b>							
Nano RR	0.1 x 150	3.5	5065-9910				
Nano RR	0.075 x 150	3.5	5065-9911				
Nano RR	0.075 x 50	3.5	5065-9924	5065-9923			
Trap/guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914			
Trap/guard hardware kit			5065-9915	5065-9915			