

# Organic Solvent and Size Exclusion



Chad Eichman, Ph.D.  
BioPharm Global Marketing Manager

### How do I optimize my SEC separations using organic solvent?

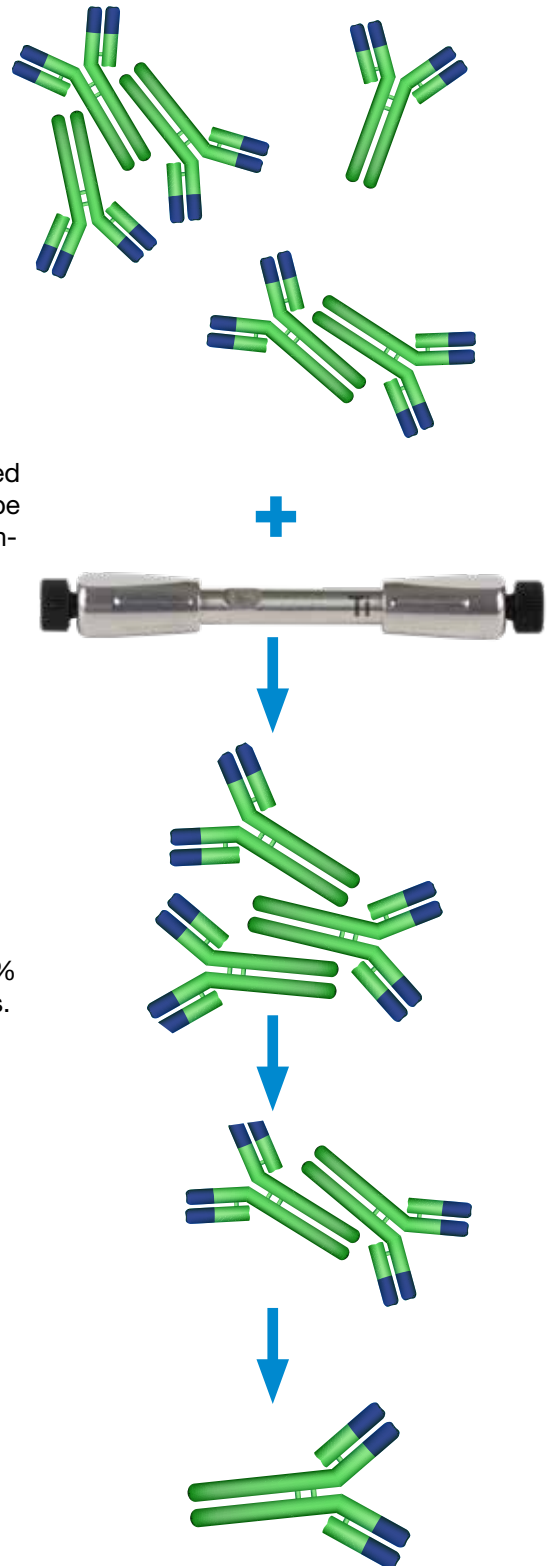
In order to achieve an optimized SEC separation (i.e. purely entropic separation, with no interaction of analyte with stationary phase), an organic modifier might be necessary. A recommended starting point for organic modifiers is 5-15 % isopropanol (IPA) or acetonitrile.

However, the question now is whether the protein is in a truly native state; one of the main contributors to aggregation are the hydrophobic interactions between monomers and fragments.

Most methods for antibody-drug conjugates (ADCs) use some organic solvent, with 15 % IPA being the most common. This is widely accepted as appropriate for assessing aggregate, though results might need to be confirmed with an orthogonal sedimentation velocity analytical ultracentrifugation (SV-AUC).

### How should a column be cleaned if it is typically used to analyze protein samples?

If strong ionic interactions between proteins and the stationary phase are suspected, then start cleaning with a denaturant such as 6 M guanidine hydrochloride or 10 % DMSO. If the protein is relatively hydrophobic, start by flushing out buffer with 95-100 % water, then clean out the hydrophobic proteins with a gradient from 95 % water/5 % acetonitrile up to 5 % water/95 % acetonitrile over 3-5 column volumes. During each step, be mindful that backpressures do not exceed the recommended limits; therefore adjust flow rates as necessary.



# Product Ordering Information

## bioZen™ Products - Powered by Biocompatible Hardware

bioZen Columns (mm)								Biocompatible Guard Cartridges*		
	Phases	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	150 x 4.6	300 x 4.6	for 2.1 mm	for 4.6 mm
bioZen 2.6 µm Glycan	—	00D-4773-AN	00F-4773-AN	—	—	—	—	—	AJO-9800	—
bioZen 1.6 µm Peptide PS-C18	00B-4770-AN	—	00F-4770-AN	—	—	—	—	—	AJO-9803	—
bioZen 3 µm Peptide PS-C18	—	—	—	—	00B-4771-E0	00F-4771-E0	—	—	—	AJO-7606
bioZen 1.7 µm Peptide XB-C18	00B-4774-AN	—	00F-4774-AN	—	—	—	—	—	AJO-9806	—
bioZen 2.6 µm Peptide XB-C18	00B-4768-AN	—	00F-4768-AN	00G-4768-AN	00B-4768-E0	00F-4768-E0	—	—	AJO-9806	AJO-9808
bioZen 3.6 µm Intact C4	00B-4767-AN	—	00F-4767-AN	—	00B-4767-E0	00F-4767-E0	—	—	AJO-9809	AJO-9811
bioZen 3.6 µm Intact XB-C8	00B-4766-AN	—	00F-4766-AN	—	00B-4766-E0	00F-4766-E0	—	—	AJO-9812	AJO-9814
bioZen 1.8 µm SEC-2	—	—	—	—	—	00F-4769-E0	00H-4769-E0	—	—	AJO-9850
bioZen 1.8 µm SEC-3	—	—	—	—	—	00F-4772-E0	00H-4772-E0	—	—	AJO-9851

\*AJO-7606 requires guard holder KJO-4282. All other guard cartridges require guard holder AJO-9000.



**Have Questions? Visit:**  
[www.phenomenex.com/bioZen](http://www.phenomenex.com/bioZen)  
 We're here to help!

guarantee

If bioZen columns in this technical note do not provide at least equivalent separations as compared to a competing column of the same phase, particle size, and dimensions, return the column with the comparative data within 45 days for a FULL REFUND.

### Australia

t: +61 (0)2-9428-6444  
 f: +61 (0)2-9428-6445  
[auinfo@phenomenex.com](mailto:auinfo@phenomenex.com)

### Austria

t: +43 (0)1-319-1301  
 f: +43 (0)1-319-1300  
[anfrage@phenomenex.com](mailto:anfrage@phenomenex.com)

### Belgium

t: +32 (0)2 503 4015 (French)  
 t: +32 (0)2 511 8666 (Dutch)  
 f: +31 (0)30-2383749  
[beinfo@phenomenex.com](mailto:beinfo@phenomenex.com)

### Canada

t: +1 (800) 543-3681  
 f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)

### China

t: +86 400-606-8099  
 f: +86 (0)22 2532-1033  
[phen@agela.com](mailto:phen@agela.com)

### Denmark

t: +45 4824 8048  
 f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Finland

t: +358 (0)9 4789 0063  
 f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### France

t: +33 (0)1 30 09 21 10  
 f: +33 (0)1 30 09 21 11  
[franceinfo@phenomenex.com](mailto:franceinfo@phenomenex.com)

### Germany

t: +49 (0)6021-58830-0  
 f: +49 (0)6021-58830-11  
[anfrage@phenomenex.com](mailto:anfrage@phenomenex.com)

### India

t: +91 (0)40-3012 2400  
 f: +91 (0)40-3012 2411  
[indiainfo@phenomenex.com](mailto:indiainfo@phenomenex.com)

### Ireland

t: +353 (0)1 247 5405  
 f: +44 1625-501796  
[eireinfo@phenomenex.com](mailto:eireinfo@phenomenex.com)

### Italy

t: +39 051 6327511  
 f: +39 051 6327555  
[italiainfo@phenomenex.com](mailto:italiainfo@phenomenex.com)

### Luxembourg

t: +31 (0)30-2418700  
 f: +31 (0)30-2383749  
[nlinfo@phenomenex.com](mailto:nlinfo@phenomenex.com)

### Mexico

t: 01-800-844-5226  
 f: 001-310-328-7768  
[tecnicomx@phenomenex.com](mailto:tecnicomx@phenomenex.com)

### The Netherlands

t: +31 (0)30-2418700  
 f: +31 (0)30-2383749  
[nlinfo@phenomenex.com](mailto:nlinfo@phenomenex.com)

### New Zealand

t: +64 (0)9-4780951  
 f: +64 (0)9-4780952  
[nzinfo@phenomenex.com](mailto:nzinfo@phenomenex.com)

### Norway

t: +47 810 02 005  
 f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Portugal

t: +351 221 450 488  
 f: +34 91-413-2290  
[ptinfo@phenomenex.com](mailto:ptinfo@phenomenex.com)

### Spain

t: +34 91-413-8613  
 f: +34 91-413-2290  
[esinfo@phenomenex.com](mailto:esinfo@phenomenex.com)

### Sweden

t: +46 (0)8 611 6950  
 f: +45 4810 6265  
[nordicinfo@phenomenex.com](mailto:nordicinfo@phenomenex.com)

### Switzerland

t: +41 61 692 20 20  
 f: +41 61 692 20 22  
[swissinfo@phenomenex.com](mailto:swissinfo@phenomenex.com)

### United Kingdom

t: +44 (0)1625-501367  
 f: +44 (0)1625-501796  
[ukinfo@phenomenex.com](mailto:ukinfo@phenomenex.com)

### USA

t: +1 (310) 212-0555  
 f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)

### All other countries Corporate Office USA

t: +1 (310) 212-0555  
 f: +1 (310) 328-7768  
[info@phenomenex.com](mailto:info@phenomenex.com)

**Terms and Conditions**  
 Subject to Phenomenex Standard Terms & Conditions, which may be viewed at [www.phenomenex.com/TermsAndConditions](http://www.phenomenex.com/TermsAndConditions).

#### Trademarks

bioZen is a trademark of Phenomenex.

FOR RESEARCH USE ONLY. Not for use in clinical diagnostic procedures.

© 2018 Phenomenex, Inc. All rights reserved.

 **phenomenex**  
 ...breaking with tradition™