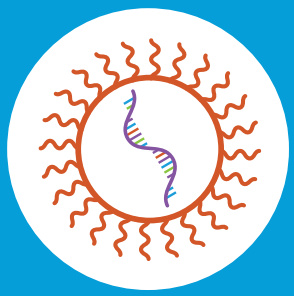
Gene Therapy Applications Guide

Advancing Gene Therapies with Confidence

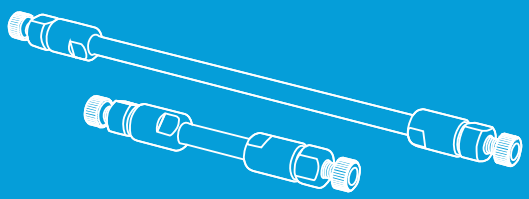
The rapid growth of gene therapy modalities—AAVs, mRNA, CRISPR, and oligonucleotides—marks a new frontier in precision medicine. As these therapies advance, the demand for robust analytical control strategies has never been greater. Reliable, reproducible testing is critical to ensure the quality, safety, and efficacy of these groundbreaking treatments.

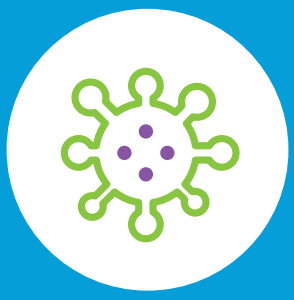
Our cutting-edge chromatographic solutions empower you to characterize complex modalities with precision, from capsid integrity to mRNA purity and beyond. With tools designed to meet the evolving challenges of gene therapy development, you can confidently accelerate innovation while maintaining control every step of the way. Tomorrow's breakthroughs demand today's solutions.

mRNA



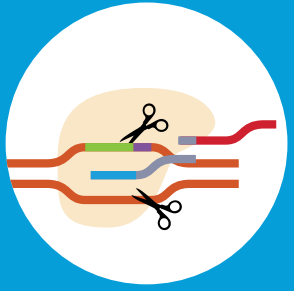
LNP





Viral Vectors

Oligos



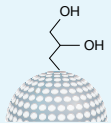
CRISPR

Contents

Select The Appropriate Fit-For-Purpose Chemistry

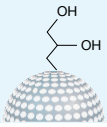
Selecting the appropriate fit-for-purpose chemistry is crucial for the accurate analysis and characterization of biomolecules. Phenomenex offers a comprehensive range of high-performance columns designed to meet your specific gene therapy research needs, ensuring reliable and reproducible results.

SEC



Biozen dSEC-7
3 µm

Inert, 700 Å large pore particle for the separation and quantitation of AAVs, IgMs and other large biotherapeutic aggregate and fragment analysis.



Biozen dSEC-2
1.8 µm and 3 µm

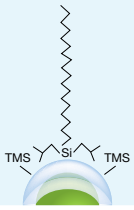
Inert, high-strength porous particle for the separation and quantitation of monoclonal antibody aggregate and fragments.

Biozen SEC-3
1.8 µm

Extremely inert, high density fully porous particle with high efficiency and high molecular weight (HMW) separation range of 10 k–700 kDa.

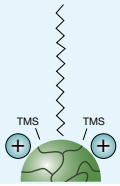
Biozen™ SEC columns feature a broad range of pore sizes and surface chemistries designed to reduce sample consumption and method times. These complementary chemistries ensure efficient and comprehensive characterization of viral vectors, large biomolecules, and their subcomponents.

Peptide



Biozen Peptide XB-C18
1.7 µm and 2.6 µm

Overall retention of both acidic and basic peptides through C18 stationary phase with di-isobutyl side chains.

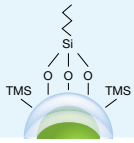


Biozen Peptide PS-C18
1.6 µm and 3 µm

Excellent retention by combined positively charged surface ligand and C18 ligand.

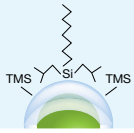
Biozen Peptide XB-C18 and PS-C18 feature complementary selectivities and inert hardware designed to maximize resolution and sequence coverage. These columns ensure exceptional peak capacity and sensitivity, enabling detailed analysis of therapeutic peptides essential for gene therapy development.

Intact



Biozen WidePore C4
2.6 µm

Core-shell particle with butyl stationary phase and optimal wide pore size distribution for improved resolution of large biologics, including monoclonal antibodies and subunit analysis.

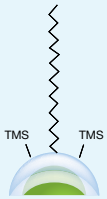


Biozen Intact XB-C8
3.6 µm

Large pore core-shell particle for fast intact and subunit biologic mass transfer. C8 provides highly useful moderate hydrophobic selectivity.

Biozen WidePore C4 and Intact XB-C8 columns are designed for high-resolution analysis of intact proteins and large biomolecules. These columns provide robust performance and reproducibility, essential for the characterization of monoclonal antibodies and other large biomolecule complexes.

Oligonucleotides

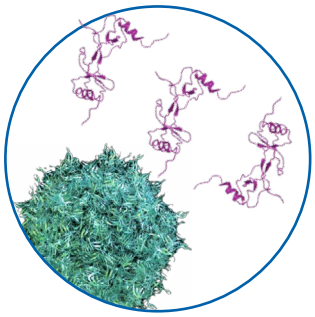


Biozen Oligo
1.7 µm and 2.6 µm

Organo-silica core-shell particle bonded with a C18 stationary phase offers high selectivity for even minute oligo differences alongside high and low pH robustness.

Biozen Oligo columns are tailored for the analysis of oligonucleotides. Featuring bioinert hardware designed to mitigate non-specific interactions and maximize recovery, high-efficiency separations and excellent reproducibility are achieved to support the development of nucleic acid-based therapeutics.

Applications



Intact Identification of Adeno-Associated Virus Capsid Proteins

AAV vectors are pivotal in gene therapy due to their broad tissue tropism and diverse serotypes. These serotypes share structural proteins—VP1, VP2, and VP3—critical for capsid formation. Given the high homology of these proteins, precise characterization of post-translational modifications are essential for understanding vector functionality. In this study, we use LC-MS with a Biozen™ Intact XB-C8 column to profile denatured and dissociated capsid proteins from AAV5 and AAV9. This method enables detailed analysis, including identification of the covalent VP1-VP2 complex in AAV9 upon denaturation without reduction. Such insights are critical for evaluating AAV quality and ensuring the consistency of gene therapy vectors.

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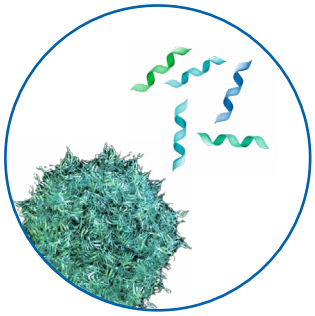

AAV Capsid Protein Peptide Mapping by Analytical and Micro Flow RPLC-HRMS

Peptide mapping is critical for assessing the structure, modifications, and integrity of viral proteins. This study highlights the benefits of microflow LC-HRMS over conventional analytical flow, delivering 98 % peptide sequence coverage with a tenfold increase in ion sensitivity, peak area, and S/N ratio. Microflow also reduces sample requirements by 95 % while improving MS2 data quality and ion intensities. These advantages enhance sensitivity, sequence coverage, and efficiency, enabling more reliable protein analysis for AAV-based therapeutics.

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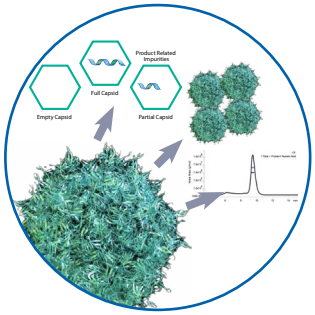

Assessing CQAs of AAVs Including Aggregation and Capsid Load Using Biozen dSEC-7

Accurate characterization of AAV therapeutics is critical for ensuring product quality, safety, and efficacy. This application note highlights the ability of Biozen dSEC-7, coupled with multi-angle light scattering (MALS), to reliably resolve monomers and aggregates from two AAV serotypes. The method enables precise determination of capsid and DNA molar masses, particle concentration, and the percentage of full AAV capsids. These insights are essential for assessing AAV purity, particle integrity, and formulation quality, supporting the development of robust and effective gene therapy products.

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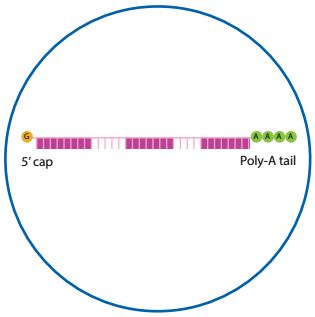
Applications



New Methods for Determining CQAs for mRNA by Enzymatic Digestion and LC-MS/MS Analysis

mRNA therapeutics require robust analytical methods to assess purity, stability, and sequence integrity. The 5' Cap and Poly(A) tail are critical features, enhancing mRNA stability and improving translation, yet contaminants such as degradation products and incomplete capping can impact therapeutic efficacy. This application note highlights a workflow utilizing enzymatic digestion followed by LC-HRMS using a Biozen™ Oligo column. This method enables precise mRNA sequence mapping in addition to quantitation of the 5' Cap and Poly(A) tail, providing a comprehensive solution for evaluating mRNA integrity and quality.

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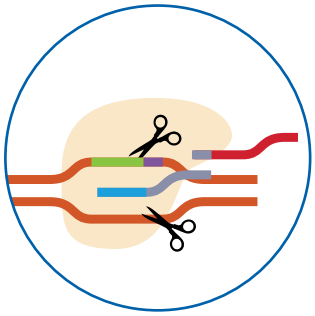

Analysis of Cas9 IVT mRNA Aggregates by Size Exclusion Chromatography Using Biozen dSEC-7

Accurate characterization of Cas9 IVT mRNA, a key component in gene-editing therapeutics, is essential for ensuring product efficacy. This application note demonstrates the capabilities of the large-pore Biozen dSEC-7 column in separating and quantifying 4500-nucleotide Cas9 mRNA and its aggregates, offering a reliable solution for quality assessment and aggregate control in mRNA-based therapies.

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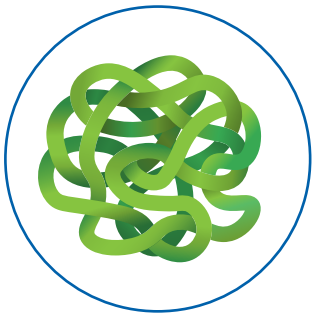

EGFP IVT mRNA Aggregation by Size Exclusion Chromatography Using Biozen™ dSEC-7

mRNA stability is critical for the efficacy of vaccines and therapeutics, requiring precise aggregate analysis. This application note highlights the Biozen dSEC-7 column's ability to separate mRNA aggregates under varying heat, salt, and flow conditions. With optimal performance at 100 mM sodium chloride and lower flow rates, Biozen dSEC-7 provides exceptional sensitivity and resolution, supporting the optimization of mRNA production and ensuring superior product quality.

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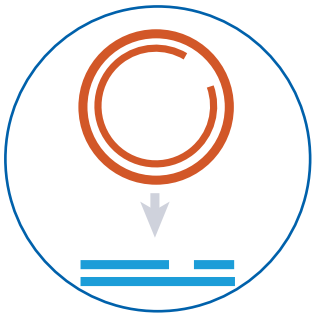
Applications



Using Biozen dSEC-7 for Plasmid Linearization In-Process Control

Linear plasmids are vital DNA templates for in vitro transcription (IVT) in mRNA therapeutic manufacturing, requiring stringent quality control to ensure proper linearization. This application note demonstrates the effectiveness of size exclusion chromatography (SEC) with the Biozen™ dSEC-7 column for separating linear plasmids from circular and supercoiled DNA. The method enables reliable monitoring of linearization, ensuring template purity for high-quality mRNA production.

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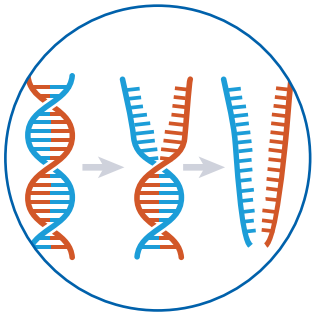

Separation of Single-Stranded and Duplex Oligonucleotides by Size Exclusion Chromatography Using Biozen dSEC-2

Precise separation of single-stranded oligonucleotides from their duplex forms is critical for quality control and therapeutic development. This application note demonstrates the effectiveness of size exclusion chromatography (SEC) in resolving oligonucleotide mixtures of varying lengths (21, 40, 80, and 100mers), providing a reliable method for characterizing these essential biomolecules.

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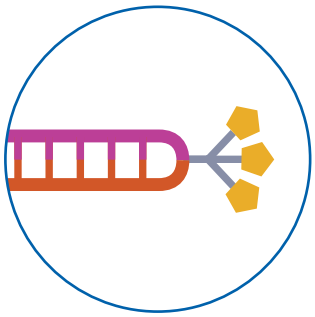

Impurity Profiling of GalNAc-conjugated Oligonucleotides by LC-MS-UV

GalNAc-conjugated siRNA duplexes are crucial for targeted gene silencing in therapeutics, but their complexity demands precise separation and impurity profiling. This application note highlights the use of ion-pairing reversed-phase LC-MS with the Biozen WidePore C4 400 Å column. By leveraging its larger pore size and optimized chemistry, this method successfully resolves siRNA strands and impurities, offering a robust solution for metabolite profiling and quality assessment of these advanced therapeutics.

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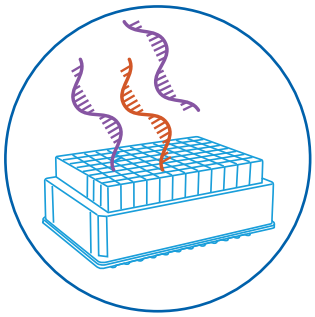
Applications



Robust Extractions of Phosphorothioate and Conjugated Oligonucleotides from Plasma and Liver Using Clarity OTX Microelution SPE

Oligonucleotide therapeutics, such as GalNAc-conjugated RNA duplexes and phosphorothioate oligonucleotides like Nusinersen, play a pivotal role in gene silencing and antisense therapies. Accurate extraction and characterization are essential for ensuring their therapeutic potency and safety. This application note demonstrates the Clarity™ OTX 2 mg/well microelution plate and Biozen™ Oligo 1.7 µm LC column's ability to deliver efficient extraction and precise analysis, supporting quality control for these critical biotherapeutics.

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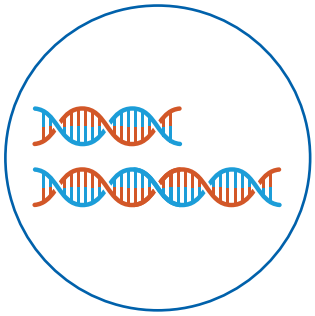

Using dSEC-7 column for Separation and Length Determination of mRNA

In vitro transcribed (IVT) messenger RNA (mRNA) has emerged as a promising new drug class for delivering genetic information, making the development of reliable analytical methods crucial. This application note presents size exclusion chromatography (SEC) as an effective method for size-based separation and length determination of mRNA. Using a Biozen dSEC-7 column, various mRNA lengths (900–10,000 nt) were analyzed, demonstrating a strong correlation between mRNA length and retention time. The method not only ensures accurate length determination but also assesses the purity of mRNA samples, highlighting its importance in the pharmaceutical analysis of biotherapeutics.

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Ordering Information

Biozen™ Products - Powered by Biocompatible Hardware

Biozen Columns (mm)										Biocompatible Guard Cartridges		
	50 x 2.1	100 x 2.1	150 x 2.1	250 x 2.1	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	300 x 4.6	for 2.1 mm	for 4.6 mm	Holder
										/3pk	/3pk	ea
Biozen 2.6 µm Glycan	00B-4773-AN	00D-4773-AN	00F-4773-AN	—	—	—	—	—	—	AJ0-9800	—	AJ0-9000
Biozen 1.6 µm Peptide PS-C18	00B-4770-AN	00D-4770-AN	00F-4770-AN	—	—	—	—	—	—	AJ0-9803	—	AJ0-9000
Biozen 3 µm Peptide PS-C18	00B-4771-AN	—	00F-4771-AN	—	00B-4771-E0	—	00F-4771-E0	—	—	AJ0-7605 *	AJ0-7606 *	KJ0-4282
Biozen 1.7 µm Peptide XB-C18	00B-4774-AN	00D-4774-AN	00F-4774-AN	—	—	—	—	—	—	AJ0-9806	—	AJ0-9000
Biozen 2.6 µm Peptide XB-C18	00B-4768-AN	00D-4768-AN	00F-4768-AN	00G-4768-AN	00B-4768-E0	—	00F-4768-E0	—	—	AJ0-9806	AJ0-9808	AJ0-9000
Biozen 2.6 µm WidePore C4	00B-4786-AN	00D-4786-AN	00F-4786-AN	—	00B-4786-E0	00D-4786-E0	00F-4786-E0	00G-4786-E0	—	AJ0-9816	AJ0-9818	AJ0-9000
Biozen 3.6 µm Intact XB-C8	00B-4766-AN	00D-4766-AN	00F-4766-AN	—	00B-4766-E0	—	00F-4766-E0	—	—	AJ0-9812	AJ0-9814	AJ0-9000
Biozen 6 µm WCX	00B-4777-AN	00D-4777-AN	00F-4777-AN	00G-4777-AN	00B-4777-E0	00D-4777-E0	00F-4777-E0	00G-4777-E0	—	AJ0-9400 *	AJ0-9401 *	KJ0-4282
Biozen 1.7 µm Oligo	00B-4791-AN	00D-4791-AN	00F-4791-AN	—	—	—	—	—	—	AJ0-9820	AJ0-9822	AJ0-9000
Biozen 2.6 µm Oligo	00B-4790-AN	00D-4790-AN	00F-4790-AN	—	00B-4790-E0	00D-4790-E0	00F-4790-E0	—	—	AJ0-9820	AJ0-9822	AJ0-9000
Biozen 1.8 µm SEC-3	00B-4772-AN	—	00F-4772-AN	—	—	00D-4772-E0	00F-4772-E0	—	00H-4772-E0	—	AJ0-9851	AJ0-9000
Biozen Native-RP-5	00B-4800-AN	—	—	—	00B-4800-E0	—	—	—	—	—	—	—

* 10/pk

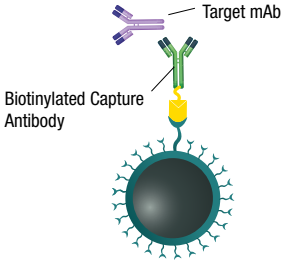
Biozen dSEC Columns (mm)							Guard Columns		
	50 x 2.1	150 x 2.1	150 x 4.6	300 x 4.6	150 x 7.8	300 x 7.8	30 x 2.1	30 x 4.6	40 x 7.8
Biozen 1.8 µm dSEC-2	00B-4787-AN	00F-4787-AN	00F-4787-E0	00H-4787-E0	—	—	—	03A-4788-E0	—
Biozen 3 µm dSEC-2	—	—	00F-4788-E0	00H-4788-E0	00F-4788-K0	00H-4788-K0	—	03A-4788-E0	03Q-4788-K0
Biozen 3 µm dSEC-7	—	00F-4789-AN	00F-4789-E0	00H-4789-E0	—	—	03A-4789-AN	03A-4788-E0	—

Sample Preparation

Biozen Solid Phase Extraction	Format	Sorbent Mass	Part Number	Unit
Biozen N-Glycan Clean-Up	Microelution 96-Well Plate	5 mg/well	8M-S009-NGA	1/box

Biozen MagBeads Streptavidin Coated

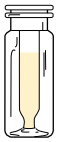
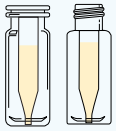
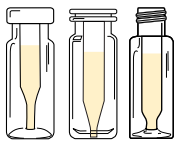
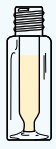
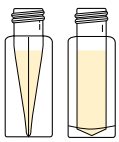
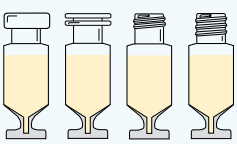
Formats	Part No.	Concentration	Bead Size
25 mg (≈50 samples)	KS0-9531	20 mg/mL	1.0 µm
50 mg (≈100 samples)	KS0-9532		
500 mg (≈1000 samples)	KS0-9533		



Ordering Information

Clarity OTX			
Part No.	**Description**		**Unit**
KS0-8494	Clarity OTX Starter Kit- Tubes	Includes: 100 mg/3 mL cartridges (x50) Lysis-loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
KS0-9253	Clarity OTX Starter Kit- 96-Well Plate	100 mg/ 96-well plate (x1) Lysis-loading buffer (100 mL) Equilibration buffer (250 mL) Wash buffer (350 mL) Elution buffer (100 mL)	ea
8M-S103-4GA	Clarity OTX Microelution Well Plate	2 mg/ well	1/box
8E-S103-CGA	Clarity OTX Well Plate	25 mg/ well	1/box
8E-S103-EGA	Clarity OTX Well Plate	100 mg/ well	1/box
8B-S103-EBJ	Clarity OTX Cartridge	100 mg/3 mL	50/box
8B-S103-HCH	Clarity OTX Cartridge	500 mg/6 mL	30/box
AL0-8579	Clarity OTX Lysis-Loading Buffer V2.0	1 L	ea

Verex 12 x 32 mm Limited Volume Specialty Vials and Kits

	Type and Description	Finish	Material	Total Volume	Residual Volume	Available as	Page Number	Part Numbers
	High-Recovery CD Vial Center-draining	9 mm Screw Thread	Glass	1.5 mL	< 20 µL	Convenience Kits (certified and regular)	37 37 36 36	AR0-9981-13 AR0-9982-13 AR0-9985-13-C AR0-9986-13-C
	Max-Recovery CD Vial Center-draining	11 mm Snap or 9 mm Screw Thread	Glass	1.5 mL	< 2 µL	Vials (regular) Convenience Kits (certified)	34 36 36	AR0-3680-12 AR0-9987-13-C AR0-9988-13-C
	Insert Vial µVial i2V	11 mm Snap	Glass	500 µL	< 2 µL	Vials (regular)	34 34	AR0-3630-13 AR0-3631-13
	Insert Vial µVial i3 (Qsert)	11 mm Snap or 9 mm Screw Thread	Glass	300 µL	< 4 µL	Convenience Kits (certified and regular)	34 34 37 37 36	AR0-9671-13 AR0-9672-13 AR0-9973-13 AR0-9974-13 AR0-9974-13-C
	Insert Vial µVial i3 (Qsert)	11 mm Crimp or 11 mm Snap or 9 mm Screw Thread	Glass	475 µL	< 4 µL	Vials (regular)	34 33 33 37 37	AR0-3625-13 AR0-3725-13 AR0-3726-13 AR0-3920-13 AR0-3921-13
	Insert Vial µVial i3 (Qsert)	10 mm Screw Thread	Glass	450 µL	< 2 µL	Vials (regular)	40 40	AR0-3020-13 AR0-3021-13
	Plastic Vial	9 mm Screw Thread	Polypropyl-ene	1.7 mL 700 µL 300 µL	< 2 µL	Vials (certified and regular)	37	AR0-39P0-13 AR0-39P1-13 AR0-39P2-13
	Micro Vial with Tapered Base v-Vial	11 mm Crimp or 11 mm Snap or 9 mm Screw Thread or 10 mm Screw Thread	Glass	1.5 mL	< 4 µL	Vials (regular)	40 34 34 33 33 37 37	AR0-3040-13 AR0-3640-13 AR0-3641-12 AR0-3740-13 AR0-3741-13 AR0-3940-13 AR0-3941-13

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