HiPer™ QA Resin

User Guide

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Abbreviations

AAV	adeno-associated virus
AEX	anion exchange
BH	bed height
BTP	Bis-Tris Propane
CIP	cleaning-in-place
cm	centimeter
CV	column volume
DBC	dynamic binding capacity
DNA	deoxyribonucleic acid
DOE	design of experiments
DTT	dithiothreitol
EDTA	ethylenediaminetetraacetic acid
g	gram
GMP	Good Manufacturing Practice
GnHCl	guanidine hydrochloride
H	height
hr	hour
ID	internal diameter
IPA	isopropyl alcohol
L	liter
LG	linear gradient
M	molar concentration
MeOH	methyl alcohol
mg	milligram
µg	microgram
$MgCl_2$	magnesium chloride
$MgSO_4$	magnesium sulfate
min	minute
mL	milliliter
µL	microliter
µm	micrometer, micron
mM	millimolar concentration
mRNA	messenger RNA
mS	millisiemen
nm	nanometer
NaCl	sodium chloride
NaOH	sodium hydroxide
OPUS	Open Platform User Specified
pI	isoelectric point
PPE	Personal Protective Equipment
RNA	ribonucleic acid
SDS	sodium dodecyl sulfate
sec	second
VG	Viral Genomes
VP	Viral Particles

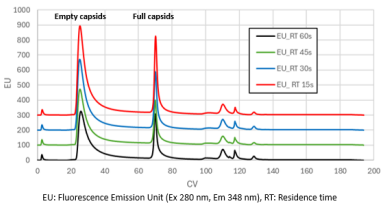
1. Introduction

Adeno-associated viruses (AAVs), plasmid DNAs, mRNAs, exosomes, and attenuated viruses and virus-like particles are gaining prominence in the advanced therapeutic paradigms, from cell and gene therapies to vaccines against cancers and microbial infections. These macromolecules are structurally complex with diverse hydrodynamic radii in the range of 20 to 150 nm, which is well beyond the mass transfer limit of traditional chromatography resins. The manufacturing feed streams often contain high levels of product-related impurities, presenting enormous challenges in obtaining highly pure drug substance from downstream processing.

HiPer™ QA Resin is an anion exchanger (AEX) built on Repligen proprietary DuloCore® particles, with unique open-pore rigid structures designed for the robust and scalable manufacturing of large biologics modalities. Target molecules are driven by convective flow to achieve efficient mass transfer and easy access to the charged ligands on particles. This chromatography resin is effective in the resolution of weak acidic species with minor difference in their isoelectric points (pI), which is ideal for applications like the separation of AAV capsids, removal of capsid protein fragments and/or aggregates, and clearance of host cell proteins and nucleic acids. It can be used for polish purification of AAVs in downstream processing, complementary to the virus capture operation with AVIPure® HiPer™ AAV Affinity Resins. OPUS® columns packed with HiPer QA Resin can be operated at high flow rates for enhanced process productivity and consistency. The separation effectiveness of HiPer QA chromatography is consistent in the residence time range of 15 to 60 seconds, as evidenced by the constant viral capsids A260/A280 readings and the elution peak area integration ratio between AAV8 empty and full capsids (Figure 1A). The process-scale OPUS columns maintain excellent pressure-flow properties, when mobile phase linear velocity is well over 1000 cm/hr (Figure 1B). They are suitable for multiple GMP production cycles, through proper cleaning-in-place (CIP) procedures, for the cost-effective manufacturing of highly pure drug materials. Detailed technical specifications of HiPer QA Resin can be found in Table 1.

Figure 1. High Speed Separation of AAV8 Empty and Full Capsids using HiPer QA Chromatography

A.

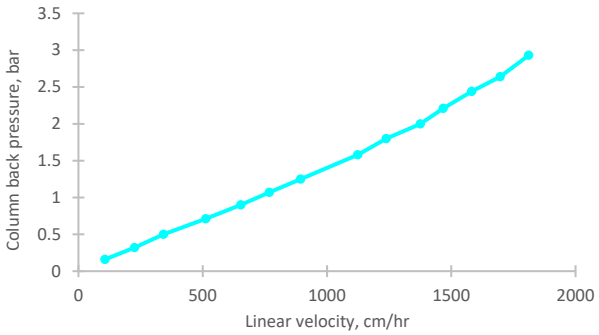


Chromatography method	
Buffer A	25 mM BTP + 10 mM $MgCl_2$, pH 8.5
Buffer B	0.2 M NaCl in Buffer A
Column stripping	Buffer B, 10 CV
CIP	1M NaOH, 10 CV
Flow rate	0.1 ~ 0.4 mL/min (1 ~ 4 CV/min)
Step elution	26.7 % Buffer B to elute empty capsids (50 CV; conductivity 9.35 mS/cm)
	(2) 50 % Buffer B to elute full capsids (30 CV; conductivity 13.9 mS/cm)
Column dimension	2.1 ID x 30 mm BH (0.1 mL)
Feedstream	AAV8 50 µL (Loading level 2E13 VP/mL resin, 20~25% full capsids by mass photometer)

EU: Fluorescence Emission Unit (Ex 280 nm, Em 348 nm), RT: Residence time

Residence time (Sec)	Empty capsid/Full capsid (Fluorescence Area %)	Empty capsid A260/280	Full capsid A260/280
60	75.67/24.33	0.58	1.29
45	76.95/23.05	0.57	1.30
30	76.59/23.41	0.55	1.30
15	75.80/24.20	0.57	1.29

B.



(A) Separation of AAV8 empty and full capsids at 15 to 60 seconds residence time.
(B) Pressure-flow property of a 10 cm ID x 10 cm BH HiPer QA OPUS column. Mobile phase 100 mM NaCl.

Table 1. HiPer QA Resin Technical Specifications

Property	Technical Specification/Description
Resin type and functional groups	Strong anion exchange, $-N^+(CH_3)_3$
Particle size	48.4 – 50.6 µm (mean)
Pore size	~1.6 µm
Dynamic binding capacity (DBC)[a]	≥10 mg/mL
pH Stability[b]	2 – 14 (1 week at ambient temperature); Avoid prolonged use at extreme pH
Shipping solution	OPUS columns in 20% (w/w) ethanol; resin slurry in 17.8% – 18.9% (w/w) ethanol
Regeneration	1 – 2 M NaCl
Sanitization	0.5 – 1 M NaOH, 30 – 60 min

Property	Technical Specification/Description
Operating flow rate	Various, depending on column configuration and chromatography station.
Maximum operation pressure	3 bar or 44 psi
Operating temperature	4°C to ambient temperature
Chemical stability	All commonly used aqueous buffers for protein purification, column cleaning solutions such as 1 M NaOH, 8 M urea, and 6 M Guanidine hydrochloride. Avoid oxidants and nucleophiles (e.g., sulfite).
Storage solutions	18% – 20% ethanol solution or 2% benzyl alcohol solution
Storage temperature	2 – 8°C

[a]10% Breakthrough capacity was determined with 1 mg/mL bovine serum albumin in 20 mM Tris-HCl (pH 8.5) at a residence time of 0.5 minute.
[b]Following exposure of test solutions, the eluting strengths of test proteins (apo-transferrin (pI ≈ 6.1), ovalbumin (pI ≈ 4.7), and soybean trypsin inhibitor (pI ≈ 4.7)) were within 99.0% to 101.5% of their original values. The DBC for bovine serum albumin was ≥90% of the original value.

2. About this Document

This manual uses several different phrases. Each phrase should draw the following level of attention:

Table 2. Explanation of User Attention Phrases

Phrase	Description
Note:	Points out useful information.
IMPORTANT	Indicates information necessary for proper instrument operation.
PRECAUTION	Cautions users of potential physical injury or equipment damage if the information is not heeded.
WARNING!	Warns users that serious physical injury can result if warning precautions are not heeded.

3. Safety Precautions

Table 3. Safety Precautions

Symbol	Description
WARNING 	Wear standard laboratory personal protective equipment (PPE), including lab coat, protective eye wear, and gloves.
WARNING 	This product is for laboratory and manufacturing production use only. Not for administration to humans.
IMPORTANT 	This product is shipped in an 20% (w/w) ethanol solution, a recognized bacteriostatic agent. It is flushed from the resin during equilibration and preparation for use. Follow all local regulations for safe disposal.
WARNING 	Flammable liquid and vapor. • Keep away from heat/spark/open flame/hot surfaces. No smoking. • Keep container tightly closed. • Ground/bond container and receiving equipment. • Store in a well-ventilated place. Keep cool.
IMPORTANT 	Dispose of contents/container in accordance with local/regional/national/ international regulations.
IMPORTANT 	For a full list of precautionary statements, please read the Safety Data Sheet (SDS).

4. Key Performance Attributes

- Macroporous matrix for the purification of large biomolecules or complexes with hydrodynamic radii in the range of 20 to 150 nm
- Resolution of closely related species, such as the empty and full capsids of AAVs at high loading level (≥1 x 10^{14} VP/mL)
- Alkaline-stable resin for ≥30 CIP cycles (30 minutes of 1 M NaOH contact time following each use)
- High recovery of target biologics and effective reduction of impurities originated from expression host cells
- Excellent particle strength and pressure-flow property, allowing operation at high flow rate for enhanced productivity
- Small elution volume for cost-effective manufacturing

5. Usage Instructions

5.1 Pre-use Sanitization

OPUS columns packed with HiPer QA Resin are shipped in 20% (w/w) ethanol solution. The resin can be sanitized with 0.5 – 1 M NaOH solution before use, if desirable. The cGMP-ready columns are pre-sterilized and certified with endotoxin and bioburden levels below 0.25 EU/mL and 10 CFU/mL, respectively. For additional information on connecting OPUS columns with specific chromatography stations, please contact customer support.

5.2 Equilibration, Binding, and Elution

Most buffers effective for maintaining pH from 4 to 9 are suitable for HiPer QA column chromatography (Table 4). The selective binding and elution of target biomolecules can be achieved by the variation of salt concentration and/or pH of buffers. Common buffer additives compatible with HiPer QA Resin (Table 5) are often useful for enhancing the removal of impurities during post-loading wash and/or facilitating the recovery of target molecules in elution. Additionally, column cleaning and regeneration can be accomplished with these common buffers. Avoid using high concentrations of anionic buffering agents or surfactants, such as phosphates, carbonates, and SDS, which may compete with the target biomolecules for positively charged ligands on the resin, thereby compromising separation efficiency.

Table 4. Common Buffers for Anion Exchange Chromatography

Buffer	pH Range
Bicine	7.6 – 9.0
Bis-Tris	5.8 – 7.2
Bis-Tris Propane	6.3 – 9.5
Diethanolamine	8.4 – 8.8
Imidazole	6.6 – 7.1
L-Histidine	5.5 – 6.0
Tricine	7.4 – 8.8
Triethanolamine	7.3 – 8.3
Tris	7.5 – 9.0

Table 5. Buffer Additives Compatible with HiPer QA Resin[a]

Chemical	Concentration
Arginine	0.5 M
Alcohols	20% to 70% ethanol, 30% IPA
Chaotropes	6 M GnHCl, 8 M Urea
Chelator	TE buffer (25 mM EDTA in 100 mM TrisHCl, pH7.0)
Divalent metals	20 mM Mg^{2+}(Cl^- or SO_4^{2-}) at pH ≤9[b]
Detergents	1% Tween® 20, 1% Tween 80, 1% Triton® X-100, 1% Triton X-114, 1% Pluronic® F68 (P188), 1% Deviron® 13-S9 (Tergital™ 15-S-9), 1% Deviron C16
Glycerol	10%
Glycine	0.5 M
Propylene glycol	10%
Reducing agents	20 mM β-mercaptoethanol, 5 mM DTT
Salts	100 mM sodium octanoate
Sucrose	1%

[a]Resin is stable in contact with chemicals for 1 week at ambient temperature.
[b]Precipitation may occur at pH >9

5.3 Regeneration, Cleaning-in-Place, and Sanitization Conditions

HiPer QA resin should be regenerated with 1 – 2 M NaCl solution after each use. A variety of reagents can be used for column cleaning-in-place, including 1 M NaOH, 25% acetic acid, 6 M GnHCl, 8 M urea, 1% Triton X-100, 6 M potassium thiocyanate, 70% ethanol, and 30% isopropyl alcohol. Used columns can be sanitized with 1 M NaOH for 30 – 60 minutes contact time. A reduced flow rate is recommended for enhancing contaminant removal and avoiding column overpressure. Following alkaline sanitization, flushing column with 1 – 2 column volumes (CV) buffer containing 1 M NaCl helps column re-equilibration.

5.4 Storage

Used columns should be fully cleaned, neutralized, and washed with at least 10 CV of deionized water before long-term storage in 18% – 20% ethanol or 2% benzyl alcohol solution at 2 – 8°C.

5.5 Chromatography Method Development

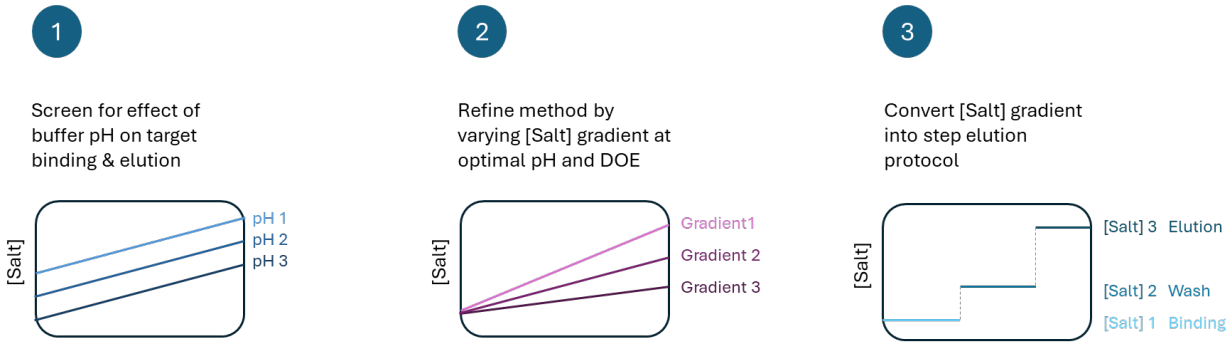
Optimal purification conditions need to be determined empirically. The interaction of a target biomolecule with HiPer QA Resin is dependent on its pI. For effective binding, separation should be conducted with a buffer with a pH at least 0.5 – 1 unit above the pI. More acidic biomolecules, such as those with lower pIs, will be retained strongly on the positively charged QA resin until elution by higher concentration of salt. Variation of buffer pH is an effective way to modulate the selectivity of binding and elution of target biologics. A schematic diagram of chromatography method development is illustrated in Figure 2A.

Fine-tuning of purification conditions can be achieved through design of experiment (DOE) studies. The resolution of empty (average pI 6.3) and full (average pI 5.9) AAV capsids on an AEX resin is typically performed at pH 7.5 – 9, through binding at low buffer conductivity followed by sequential elution at varied concentrations of NaCl[1]. The use of alternative salts and buffer additives, such as ammonium sulfate, sodium acetate, $MgCl_2$, and $MgSO_4$, has been shown to enhance the separation of full and empty capsids[1–4]. While reducing feed conductivity by extensive dilution is a common practice to promote binding on an anion exchange column, the stability of AAV capsids in low conductivity buffer, as well as lengthy processing time due to increased feed stream volume, can potentially compromise the integrity and recovery of full capsids. Purification consistency and efficiency would benefit from reduced operation time at faster flow rates to minimize the degradation of AAV capsids on column[1].

The final purity and yield of full capsids can be further impacted by titer and capsid stoichiometry in the original feed stream. Example chromatograms showing the separation of AAV8 empty and full capsids by columns packed with HiPer QA Resin are presented in Figure 2. A linear salt concentration gradient was used to assess the eluting strengths of empty and full capsids (Figure 2B). Buffers with minor variations around the empty capsid elution conductivity were tested for post-loading wash efficiency and the impact on the final purity and yield of full capsids. A buffer with conductivity of 9.35 mS/cm was selected for thorough removal of empty capsids, and an elution buffer at 13.90 mS/cm was used for recovering the desired full capsids (Figure 2C).

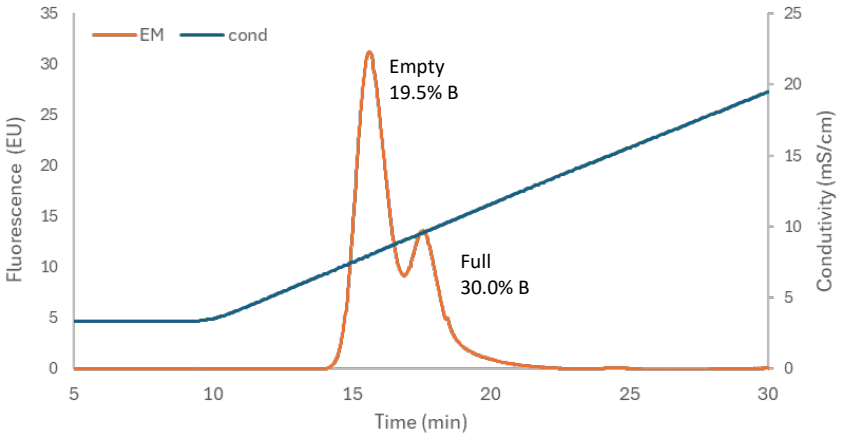
Figure 2. HiPer QA Chromatography Method Development

A. General Approach



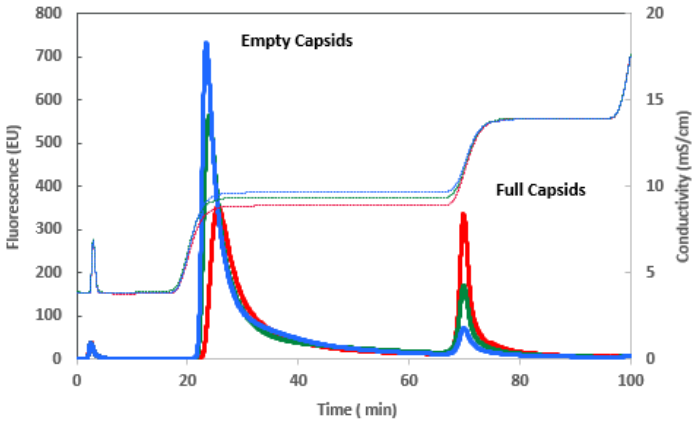
B. Separation of empty and full capsids using a linear gradient at pH 8.5

Column: 2 mm ID x 36 mm BH (0.1 mL)
Flow rate: 1 CV/min (residence time: 1 min)
Loading: AAV8 from affinity purification, 5 x 10^{11} VG/mL resin
Buffer A: 10 mM $MgCl_2$ in BTP buffer (pH 8.5)
Buffer B: Buffer A + 0.2 M NaCl
Elution: 0 – 100% B Linear Gradient in 20 CV at 1% Buffer B/min



C. Stepwise elution of empty and full capsids and effect of wash buffer conductivity on yield and purity

Column: 2 mm ID x 36 mm BH (0.1 mL)
Flow rate: 1 CV/min (Residence time: 1 min)
Loading: AAV8 from affinity purification, 5E12 VG/mL resin
Equilibration Buffer:
10 mM $MgCl_2$ in BTP buffer (pH 8.5)
Wash Buffers:
10 mM $MgCl_2$ in BTP buffer (pH 8.5) with 57.4 mM, 53.4 mM, or 49.4 mM NaCl, respectively
Elution Buffer:
10 mM $MgCl_2$ in BTP buffer (pH 8.5), 100 mM NaCl



Wash Buffer Conductivity (mS/cm)	Full Capsids Purity A260/280	Full Capsids Yield (Relative)
9.67 (57.4 mM)	1.32	0.87
9.35 (53.4 mM)	1.30	1
8.88 (49.4 mM)	1.26	1.14

6. Ordering Information

The HiPer QA resin is available in multiple OPUS column sizes for lab-scale resin screening, chromatography method development, and pilot- to manufacturing-scale downstream processing. Contact your account manager to place an order, or contact your regional customer service department using the email addresses below:

North America: customerserviceUS@repligen.com
Europe: customerserviceEU@repligen.com
China: customerserviceCN@repligen.com

Table 6. HiPer QA OPUS Column Configurations

OPUS Column	Part Number	CV	ID	BH	Notes
MiniChrom®	23010106	1 mL	0.5 cm	5 cm	For R&D screening and process development
	23010104-100	5 mL	0.8 cm	10 cm	
	23010107-100	10 mL	1.13 cm	10 cm	
ValiChrom®	23010109V-100	2 0 mL	1.6 cm	10 cm	For process validation
	23010110V-100	50	2.5 cm	10 cm	
Large Scale	Inquire[a]	25 – 800 mL	2.5 – 10 cm	5 – 10 cm	For clinical and commercial production

[a]Custom part numbers are created when the order is placed.

7. Troubleshooting

Table 7. Troubleshooting

Problem	Possible Causes	Solutions
Increase of column pressure and/or column clogging during sample loading	Target molecule or contaminants precipitate from feed stream	Check buffer compatibility with target molecule and contaminants; check solubility and stability of target molecule and contaminants; use alternative buffer if necessary; dilute feed stream with additional buffer; use appropriate buffer additives to improve solubility of target and/or contaminants. Apply upflow to the column to remove clogging materials; resanitize column in upflow if necessary.
	Feed stream clarification is insufficient	Clarify feed stream with a 0.22 μm filter.
Target molecule in flowthrough fractions	Column saturation	Reduce feed stream loading volume.
	Feed stream conductivity and/or pH is incorrect	Match feed conductivity and pH with those of equilibration buffer. Check if buffer pH is at least 0.5 to 1 unit above the pI of target molecule. Reduce the conductivity of feed stream and equilibration buffer.
Low binding capacity of target molecule	The conductivity of feed stream and equilibration buffer is too high	Reduce conductivity of feed stream and equilibration buffer. **Note:** Check the stability of target molecule and/or contaminants at lower conductivity.
	The pH of buffer and feed stream is not optimal	Perform purification at different pH values and evaluate the impact on target binding capacity and selectivity. Use buffer additives that can enhance charge interactions (i.e., propylene glycol).
Unable to elute bound target molecule	Target molecule is tightly bound	Use a more stringent elution condition, such as higher NaCl concentration; include appropriate buffer additives and/or variation of buffer pH to improve elution efficiency
Increase of column pressure during elution	Target molecule concentration in eluate is too high; target molecule precipitates	Ensure column back pressure does not exceed 3 bar or 44 psi. Reduce flow rate during elution step; include buffer additives to improve the solubility and stability of target molecule.
On-column aggregation/ precipitation of biomolecules	Biomolecule is not stable or not compatible with buffers used in downstream processing	Use alternative buffers and/or buffer additives to improve the stability of biomolecule during separation
Contaminants co-elute with target molecule	Contaminants have similar biochemical properties; Contaminants interact with target molecule	Use buffer additives to further differentiate target molecule and contaminants; use appropriate detergents or reducing agents to dissociate contaminants from target molecule. Test the effect of buffer pH on elution selectivity
Column drying out during use or storage	Air is accidentally pumped into column or column is not properly sealed during storage	Pump at least 3 CV of 5% to 20% ethanol through the column until A280 returns to baseline. Rinse column with 0.1 M NaCl followed by equilibration buffer. For ValiChrom and larger columns, perform a column performance test to ensure column integrity is restored.
Column discoloration after use	Retention of metal ions or colored organic compounds (e.g., Fe^{2+}/Fe^{3+}, Cu^{2+}, Vitamin B12, folic acid, etc.)	Strip metal ions off the column with 5 – 10 mM EDTA. 5% to 20% ethanol can be used to remove bound organic compounds. Rinse column with 0.1 M NaCl followed by H_2O (optional) and re-equilibrate the column.
Loss of AAV capsids in flowthrough fractions	The apparent pI of AAV capsids is different from prediction due to post-translational modifications and/or culturing conditions. Denaturation of AAV feed due to excessive dilution	Adjust buffer conductivity and pH; consider using buffer additive to improve AAV capsid stability. Minimize feed stream dilution to prevent AAV capsid aggregation at low buffer ionic strength.

Problem	Possible Causes	Solutions
Incomplete separation of AAV empty/full capsids	Insufficient wash to eliminate empty capsids before the elution of full capsids; suboptimal buffers	Extend the duration of post-loading wash; vary the conductivity and/or pH of wash buffer (e.g., higher conductivity and/or lower pH); use buffer additives (e.g., $MgSO_4$, $MgCl_2$) to enhance the resolution of empty and full capsids.
Degradation and/or denaturation of AAV capsids on column	Buffer composition, conductivity, and/or pH are not optimal; prolonged chromatography operation; presence of proteases	Screen for optimal buffer composition, conductivity, and/or pH; increase flow rate to minimize processing time and streamline process steps; identify contaminating proteases, if possible, and screen for condition that minimizes proteolytic activity.

8. References

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9. Index

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