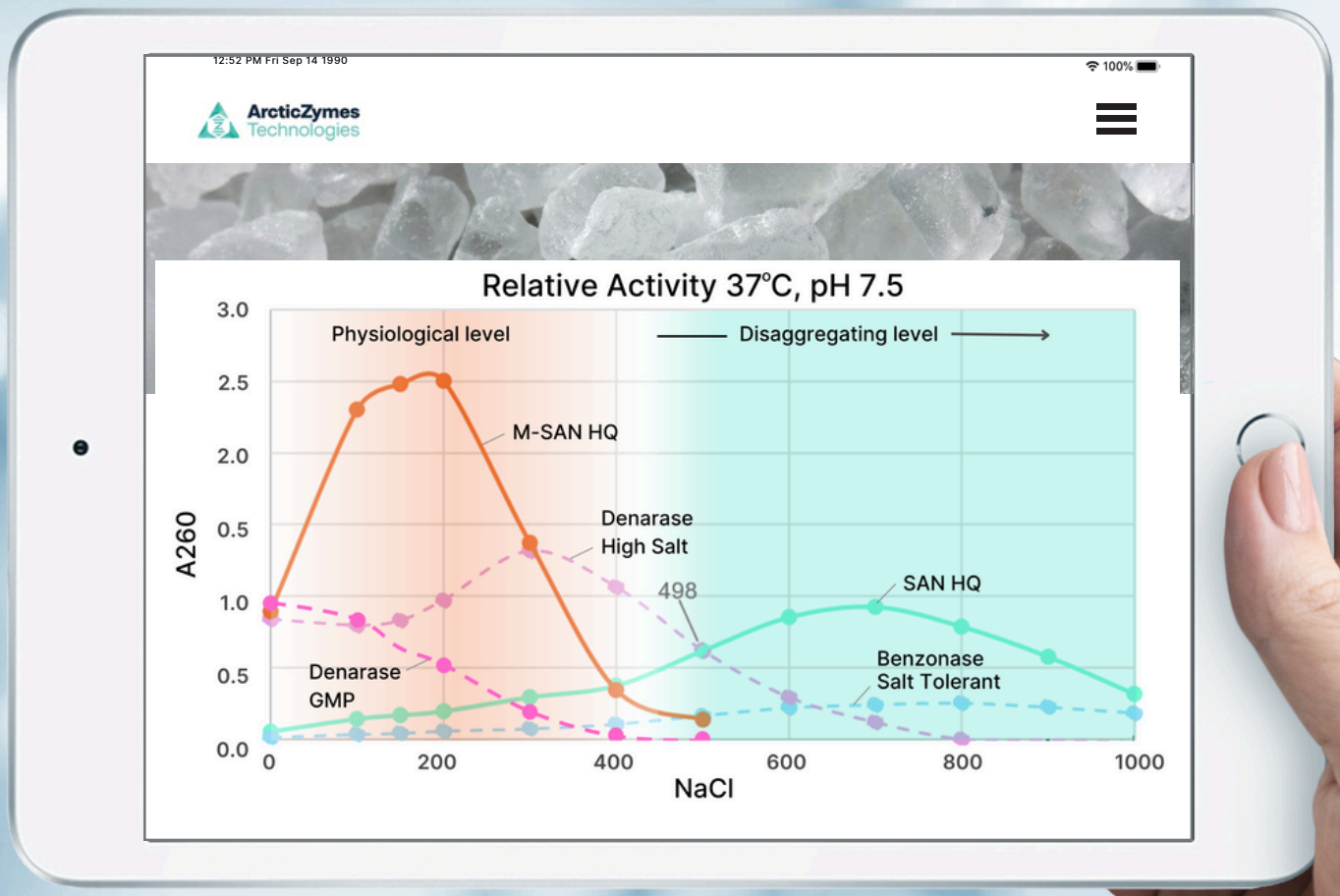
Peak Performance Where It Matters

How to maximize removal of chromatin-associated DNA



You'll learn about:

- Advantages of salt to optimize bioprocessing
- How to achieve 3 to 5x higher nuclease activity in typical cell media conditions
- The hidden chromatin contamination issue that QA and process developers often overlook

SAN HQ now in GMP Grade

ArcticZymes Technologies

Inventor of Salt Active Nucleases

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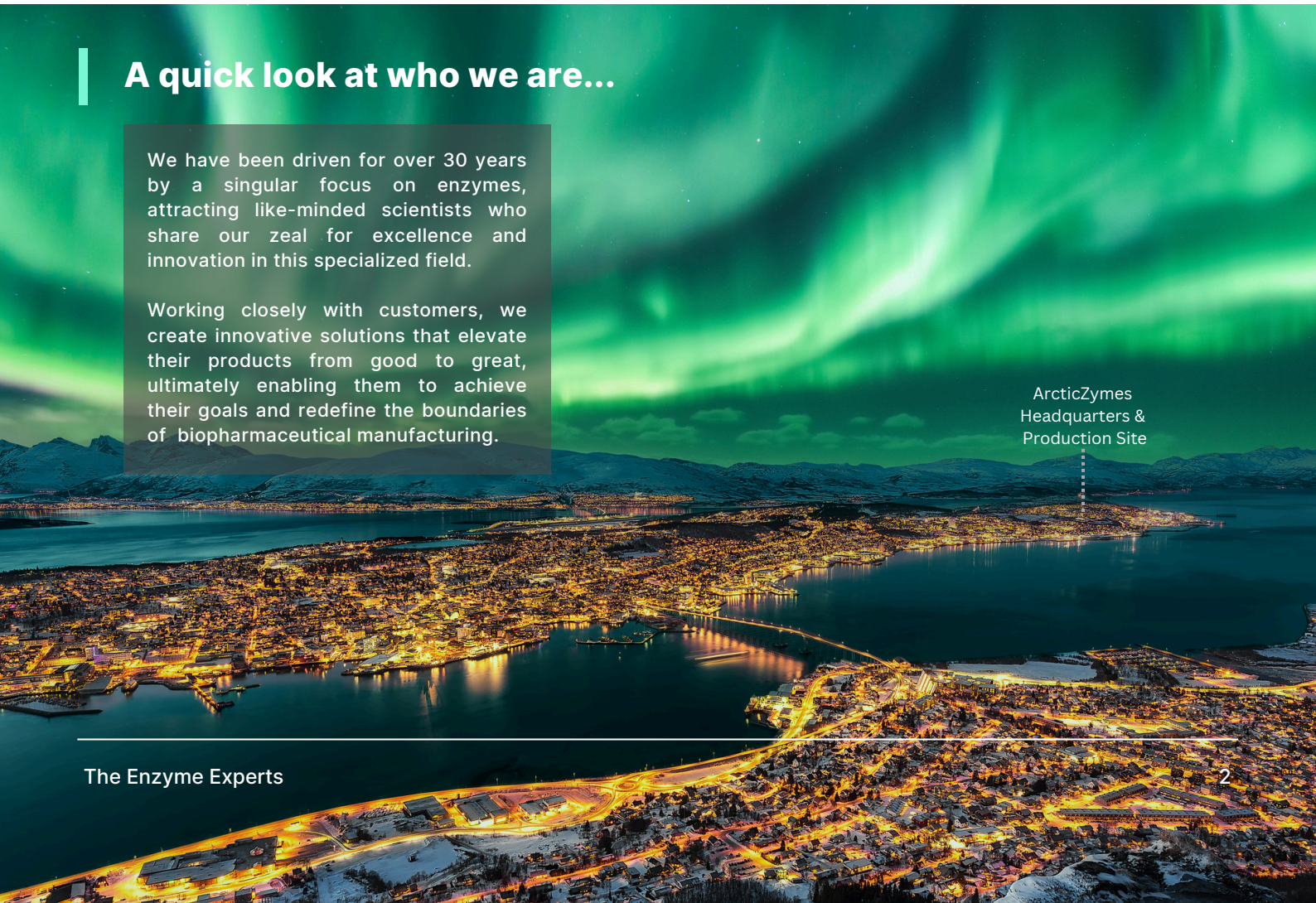
A quick look at who we are...

We have been driven for over 30 years by a singular focus on enzymes, attracting like-minded scientists who share our zeal for excellence and innovation in this specialized field.

Working closely with customers, we create innovative solutions that elevate their products from good to great, ultimately enabling them to achieve their goals and redefine the boundaries of biopharmaceutical manufacturing.



Why Salt is the Hidden Catalyst in Bioprocessing Optimization

Every organism has mastered the art of maintaining a precise salinity balance to ensure its survival. Similarly, in bioprocessing, we utilize host cells at their unique salinity preference to develop transformative therapeutic products for human well-being. Maintaining physiological salt conditions is key to keeping both host cells alive and manufacturing processes effective.

Yet, a misconception persists: salt is seen as a bioprocessing hindrance rather than an asset. We aim to demonstrate how salt is an underestimated catalyst in the quest to gain greater control and efficiency in your bioprocessing workflow.

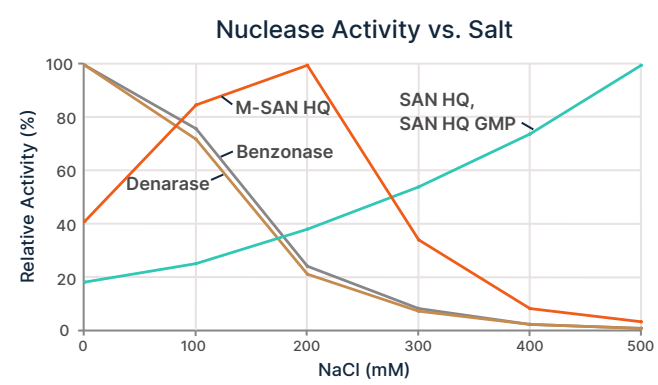
Stop adapting your process to the nuclease when... ...you can adapt the nuclease to your process!

By customizing the nuclease to fit these high salt environments, you can gain not only greater control and efficiency but also achieve higher purity, reduced interference, and enhanced cost-effectiveness with less nuclease used.

As you increase salt concentration to optimum levels, conditions for DNA clearance improve, but enzyme function decreases. This is a paradox that ArcticZymes effectively resolves by maintaining robust enzymatic activity where it matters most.

Imagine, choosing the nuclease that performs best, based on your conditions and your process, improving: yield, overall product quality, workflow simplification, and improved efficiency of DNA removal from your lysates or supernatant in a single step.

These specialized nucleases are a paradigm shift in the current landscape of available endonucleases for viral bioprocessing.



Relative Activity of Nucleases Across Salt Concentrations

SAN HQ and M-SAN HQ demonstrate high activity levels adapted to relevant salinity. In contrast, Benzonase and Denarase show diminished activity as salt concentration increases. The data provides insights into the salt-tolerance capabilities of these nucleases, crucial for optimizing bioprocessing workflows.

Turning previous challenges into advantages

For enzymatic removal of DNA impurities in bioprocessing we developed M-SAN HQ and SAN HQ to use what was previously an obstacle into achieving a revolution in cleaner viral vectors, or protein-based therapeutic products. As enzyme experts, our mission is to develop solutions that solve your challenges.

These novel nucleases maximize results at your optimum salt levels, instead of being inhibited by them.

M-SAN HQ : Optimal for the isotonic / physiological salinity found in cell media.
SAN HQ : Salt-loving nuclease optimal when increasing salt beyond isotonic, and now

INTRODUCING **SAN HQ GMP**

From the inventors of Salt Active Nucleases

Where GMP-grade meets innovation from the pioneers salt-active nucleases

Why Salt Active Nucleases Are Vital to Biopharmaceutical Manufacturing

Nucleases play a critical role in gene therapy and vaccine production by digesting extraneous genetic material. Their function in DNA clearance helps meet the stringent purity requirements of therapeutic products. DNA from host cells, considered an impurity, is typically removed by adding nucleases to the isotonic cell media. Salt can be added to prevent aggregation, which can improve accessibility of the host-cell DNA and simplify the downstream purification process.

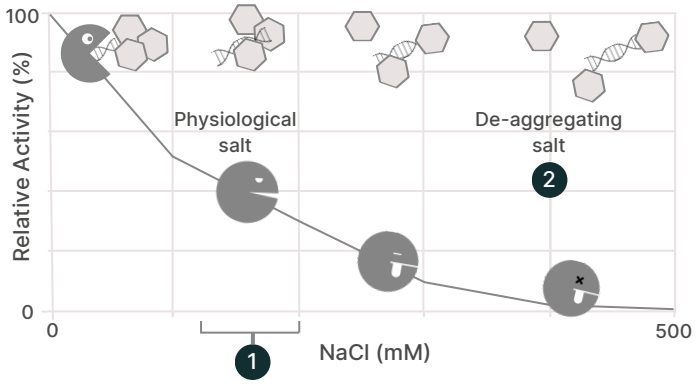
Biopharma's Untapped Potential: The Barrier to Higher Performance

THE PROBLEM: Conventional nucleases lose their ability to bind or cleave DNA at physiological salt and above.

1. Fragile vectors like Lentiviruses are typically kept at the physiological salt range found in cell media.
2. Adenoviruses and AAV's are examples of robust viral vectors prone to aggregation. Employing elevated salt concentration prevents aggregation and makes DNA more accessible for digestion.

Conventional nucleases have decreased function at cell media salt levels, and are near non-functional at de-aggregating levels of salt.

Relative Activity of **Commonly Used Nucleases**



THE CONSEQUENCES: By not utilizing salt-adapted nucleases in high-salt conditions, biopharmaceutical manufacturers **miss out on key advantages including**:

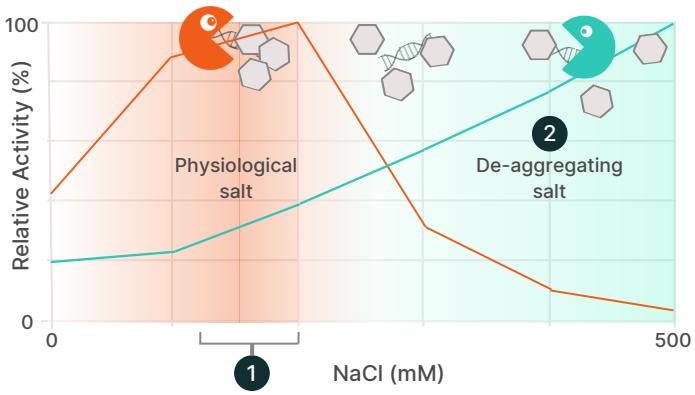
- **Improved yield**
- **Increased purity**
- **Reduced costs**
- **Streamlined workflow**

THE SOLUTION: We developed nucleases that thrive at the salt levels most relevant in bioprocessing; directly in cell media or at de-aggregating salt levels.

M-SAN HQ:
a nuclease optimized for the physiological salt range found in cell media, between 125-200 mM.

SAN HQ/SAN HQ GMP:
a nuclease optimized for high salt, enhancing its DNA-clearing functionality as salt levels rise above 300 mM.

Relative Activity of **ArcticZymes Salt Active Nucleases**



Quality Documentation

ArcticZymes is dedicated to the quality of its products and is certified according to ISO 13485.

High Purity and High Quality

As the original manufacturer of SAN HQ and M-SAN HQ nucleases, we offer full traceability of the supply chain and manufacturing process. We also assist our clients in implementing necessary identity and quality assays in-house.

Our nucleases are manufactured according to ISO 13485. In addition, relevant cGMP requirements have been implemented and the products are qualified for use by GMP-compliant customers.

The nucleases are manufactured using only non-animal origin raw materials to minimize the risk of contamination with adventitious agents.

Our manufacturing site in Norway is regularly audited by our customers.

ArcticZymes ELISA kits are used with salt active nucleases to detect residual enzyme(s).

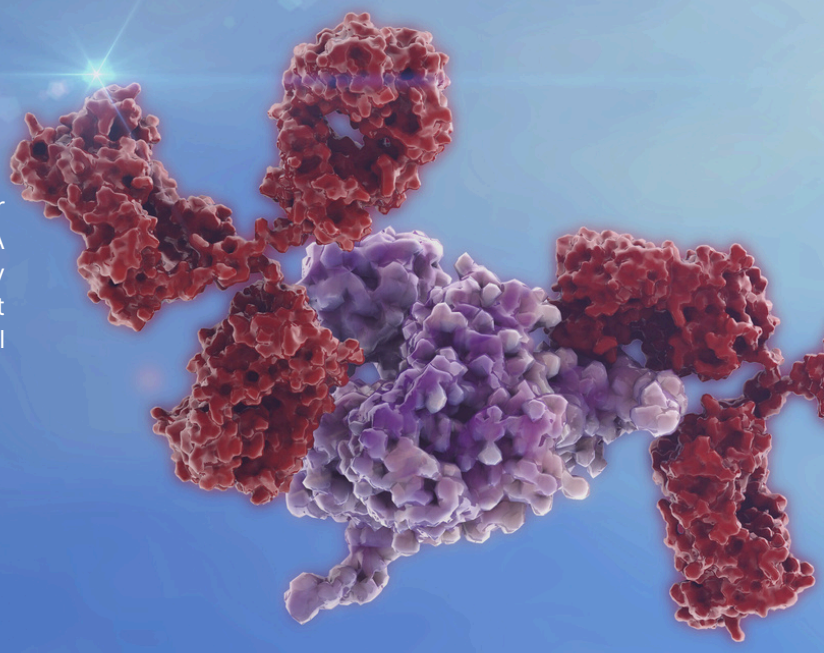
Accreditation & Quality Assurance

Examples of documentation available:

- Elemental Impurities Statement
- Residual Solvent Statement
- TSE-BSE Statement
- Latex Statement
- Allergen Certificate
- Aflatoxin Certificate
- Melamine Certificate
- GMO Certificate
- Bioburden (TYMC/TAMC)
- Endotoxin assays (EP)
- Toxicological Risk Assessment

ELISA Kit for: M-SAN HQ, SAN HQ/SAN HQ GMP

The ELISA kits for either SAN HQ/SAN HQ GMP or M-SAN HQ utilize a classical sandwich ELISA methodology and are designed to detect and quantify residual levels of your nuclease of choice, ensuring that you meet rigorous quality standards in both final products and in-process samples.



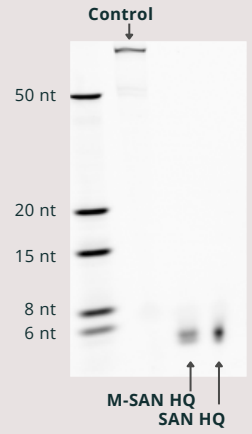
The Challenge in Removing Host Cell Chromatin Impurities

In bioprocessing, the primary role of a nuclease is to efficiently digest and fragment host-cell DNA into sufficiently small pieces, facilitating its removal during downstream processing. While most nucleases can effectively degrade naked DNA into tiny fragments under optimal conditions—as demonstrated by M-SAN HQ and SAN HQ, which can digest dsDNA into fragments smaller than 8 nt—the reality in bioprocessing is more complex.

The DNA targeted for removal often exists as chromatin, embedded in a complex matrix containing remnants of the lysed host cell as well as large amounts of the therapeutic product. The product may or may not have an affinity for the chromatin you aim to remove.

High salt is often applied to mitigate issues like aggregation. The real challenge lies in a nuclease's ability to efficiently fragment chromatin under these more complicated, high-salt, conditions—not merely degrading naked DNA under ideal circumstances.

Fragmentation of DNA at optimal conditions

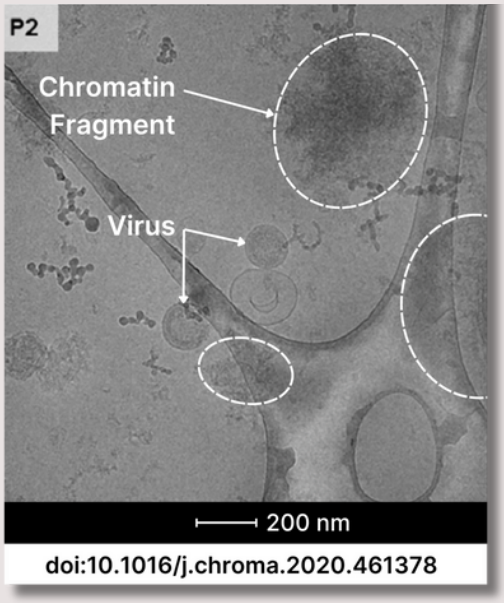


> Many researchers and bioprocess engineers are not aware that host cell DNA is present in the form of chromatin associated with histones. In the presence of salt, chromatin opens and becomes better accessible for a nuclease. Therefore, it is obvious for me that a salt-tolerant nuclease does a much better job in digesting DNA."
>
> - Professor Alois Jungbauer, University of Natural Resources and Life Sciences, Vienna

Consequences of Incomplete Chromatin Fragment Breakdown

- **Reduced Purity & Yield**
- **Increased Aggregation**
- **Regulatory Challenges**
- **Inefficient Processes**
- **Lower Product Quality**
- **Inconsistent Performance**

Large chromatin fibers left over after Benzonase digestion. Shown by Cryo-TEM Micrograph

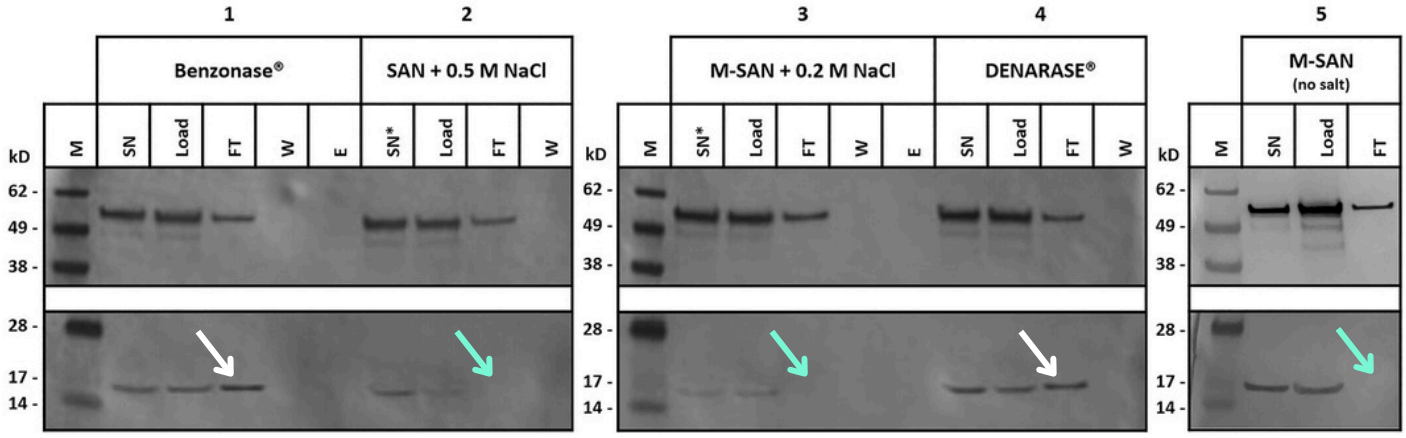


> Only M-SAN HQ and SAN HQ was degrading chromatin at a level allowing its removal in flow-through mode chromatography

Superior Chromatin Digestion Before Flow-Through Purification

In a study by Mayer et al. (2023), the use of four different nucleases were evaluated in combination with a flow-through (FT) purification chromatography method for measles viruses. The supernatant was nuclease treated prior to the FT column purification (Load) to remove DNA impurities from the viral vectors. Smaller impurities, like DNA fragments, will bind to the resin in the FT column, while the larger virus pass through and are collected in the flow-through. To evaluate the efficiency of chromatin removal, host chromatin (histone H3) was measured using western blot, shown below.

The study clearly demonstrated that M-SAN HQ and SAN HQ efficiently degrade DNA to fragments sufficiently small for capturing by the resin, resulting in **no detected chromatin in the flow-through** under all tested salt conditions, as shown by lack of band in the FT, (marked with green arrows). In contrast, for the two competitor nucleases, a clear histone H3 band is detected in the flow-through, indicating insufficient chromatin removal (marked with white arrows).



Western blots detecting viral vectors (nucleoprotein, top) and chromatin (histone H3 protein, bottom) (SN, supernatant; SN*, high salt supernatant, Load, supernatant after nuclease digestion; FT, flow-through; W, wash; E, elution). Mayer et al, Biotechnology Progress, First published: 27 March 2023, DOI:10.1002/btpr.3342

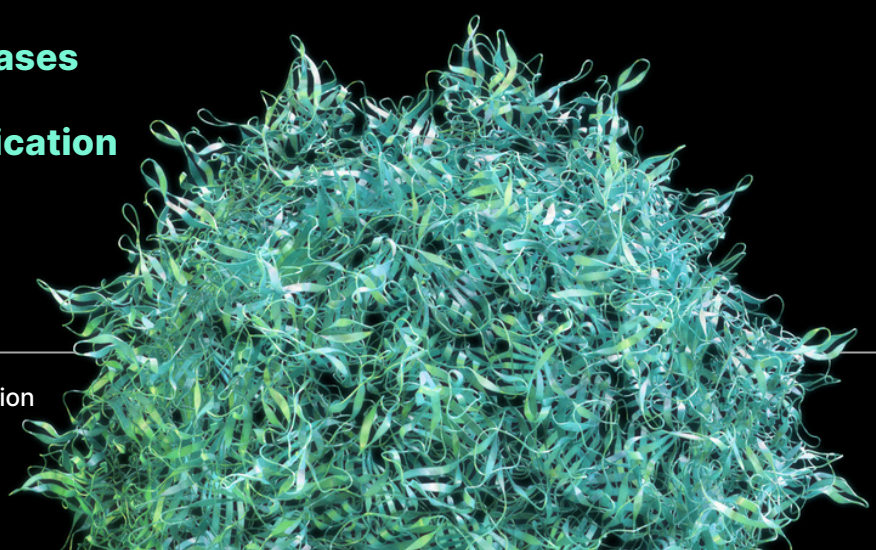
insufficient chromatin removal

no detected chromatin in flow-through

Our salt active nucleases digest DNA faster and create smaller fragments Why it matters:

- **ArcticZymes nucleases enable improved Flow-Through Purification:** Due to smaller DNA fragment size
- **Process simplification:** Reduces the need for an additional purification step
- **Reduced time, reduced cost**

ArcticZymes nucleases are ideal for Flow-through purification



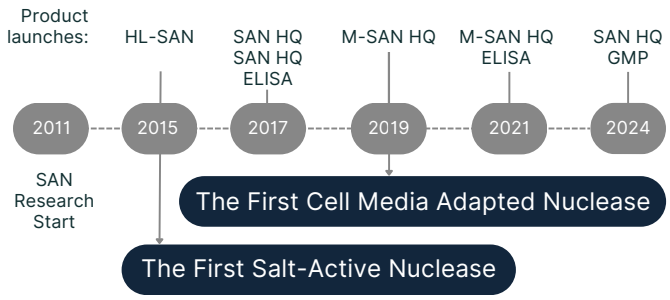
We Pioneered the First Salt Active Nucleases for Biopharma

Our research resulted in the first Salt Active Nuclease for use in Biopharmaceutical manufacturing in 2017.

By aligning with the optimal salt balance for host cells, these nucleases enhance both vitality and efficient DNA clearance, turning an obstacle into a strategic advantage. The result is a streamlined workflow, improved product quality, and a new frontier in bioprocessing efficiency.

We continue to develop nucleases that out-perform rivals and that empower you to choose the nuclease that performs best, based on your conditions and your process.

Evolution of the Salt-Active Nuclease (SAN) product family



Many design enzymes; we design novel solutions for your conditions

As scientists focused on the challenge of maximizing DNA clearance we put ourselves in your shoes and asked:

> What if,... the nuclease was designed for your ideal saline conditions, rather than you bending your process to fit the enzyme?

Enzyme expertise & innovation from the Arctic to tackle industry challenge

Situated along the Norwegian coast, 350 km north of the Arctic Circle, our lab has a passion for addressing industry-specific challenges such as enzyme efficiency in high-salt and low-temperature environments. In molecular research, diagnostics, and therapeutics, we have a proven track record of leveraging our expertise with Extremozymes:

Extremozymes:
/ɛksˈtriːmoʊˌzaɪmz/

Enzymes from extremophilic microorganisms usually catalyze chemical reactions in non-standard conditions. Such conditions promote aggregation, precipitation, and denaturation, reducing the activity of most non-extremophilic enzymes, frequently due to the absence of sufficient hydration.

A Simple Choice for Complex Processes:

For physiological salt levels, use:

M-SAN HQ
Optimal for the physiological salinity found in cell media.

M-SAN HQ ELISA kit

For high salt levels, use:

SAN HQ & SAN HQ GMP
Salt tolerant nuclease optimal with salt beyond isotonic.

SAN HQ ELISA kit

It's that simple.
Nucleases Optimized for Your Preferred Salt Levels.

> In the simplicity of nature, we find the grandest of designs.
>
> - Fridtjof Nansen, Norwegian Polar Explorer, Scientist, and Nobel Laureate

Salt crystal

M-SAN HQ: No Other Endonuclease Performs Better at Physiological Conditions

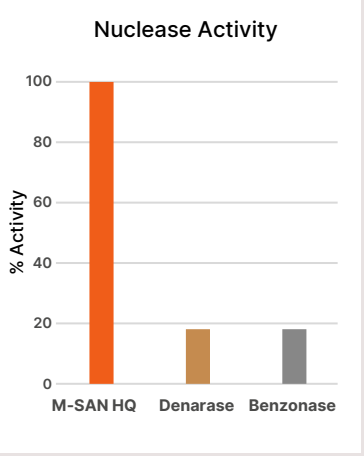
Medium-Salt Active Nuclease High Quality (M-SAN HQ) is a Bioprocessing Grade nuclease developed for removal of both single and double stranded DNA and RNA, at the physiological salt conditions most often used in bioprocessing and biomanufacturing workflows. M-SAN HQ allows you to directly replace Benzonase without changing your workflow.

Made for peak activity, where it matters most

This novel, nonspecific endonuclease demonstrates superior activity across a broad pH range. In addition, it is optimized for peak performance in a salt concentration range of 125–250 mM, a range that includes the typical physiological salt concentrations of 135–150 mM.

We engineered M-SAN HQ to peak performance at exactly the salinity range where Lentiviruses and Retroviruses are typically harvested from cell culture supernatants, thereby ensuring the highest possible results:

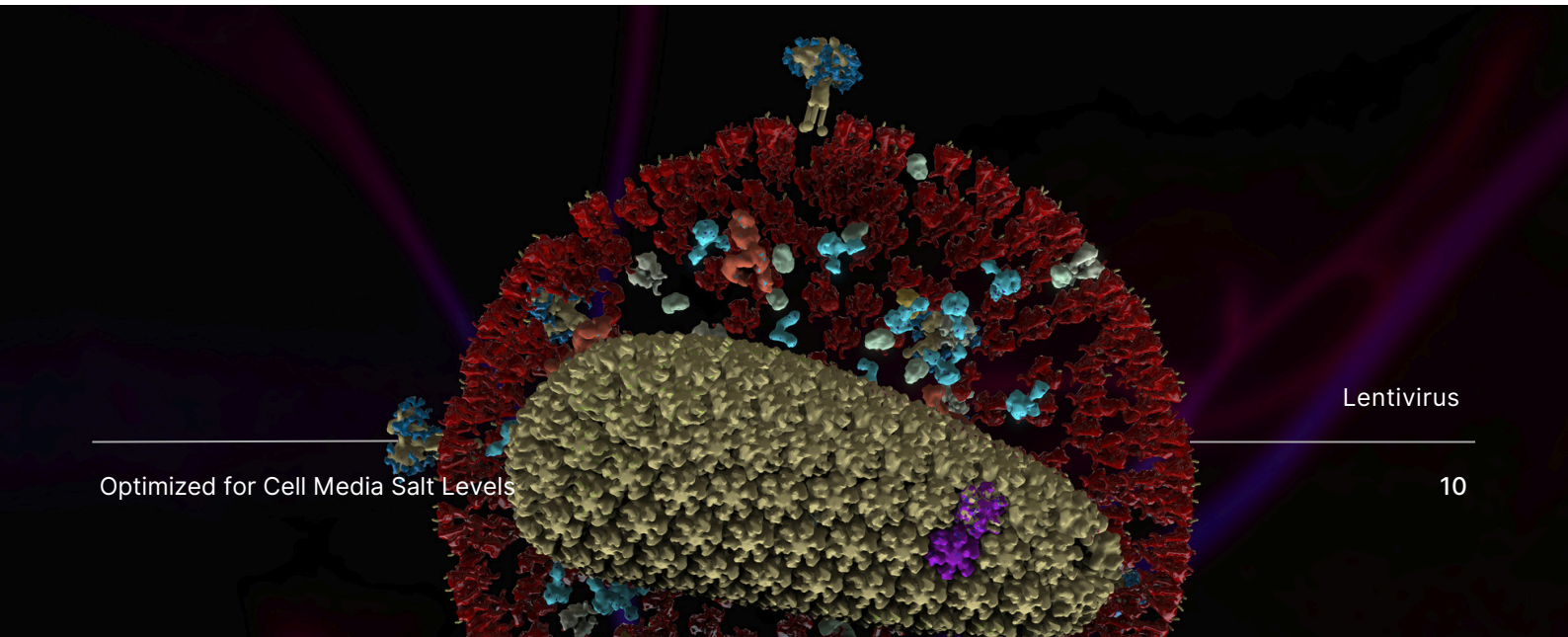
- **Compatibility:** Ideal for working with both fragile and robust viral vectors and proteins in a variety of cell media.
- **Optimum activity:** Optimization for cell media salinity allows both shorter DNA fragments and reduced incubation times.
- **Process Efficiency**: Smaller DNA fragments simplifies DSP workflows.
- **Cost-Effectiveness**: Reduction of additional reagents & steps can lower production costs.
- **Quality & Improved Yield**: Maintains the integrity of labile biological molecules, ensuring both pure and high quality end product while also improving yield.



Superior performance at physiological salinity

The activity of M-SAN HQ and often used nucleases was compared by digesting DNA at physiological salinity (150 mM NaCl), pH 7.5 @ 37°C and 5 mM $MgCl_2$. M-SAN HQ showed significant higher activity at tested conditions compared to competitor nucleases.

Applications:
Removal of nucleic acids during protein production, vaccine manufacturing and viral vector preparation.

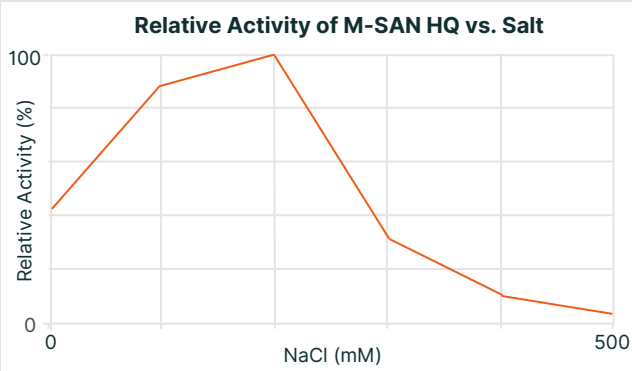


Lentivirus

Adapted to use in medium without salinity adjustments

Optimal activity at physiological salinity and pH makes it ideal for DNA removal from mammalian cell media.

The high activity of M-SAN HQ at the physiological salinity and pH found in standard cell medium conditions leads to improved DNA clearance compared to commonly used nucleases.



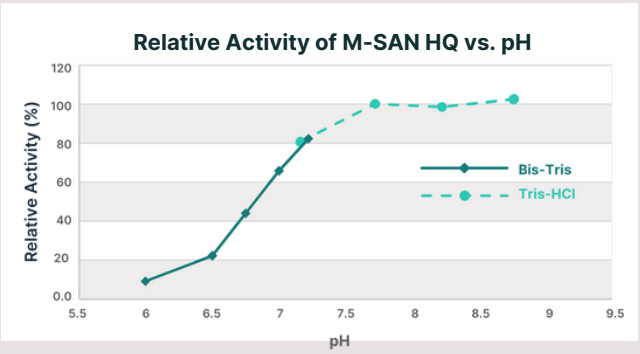
M-SAN HQ shows peak activity at physiological salt levels
Activity was measured at 37°C in a 25 mM Tris-HCl buffer at pH 7.5 @ 37°C, 5 mM $MgCl_2$ and varying concentrations of NaCl ranging from 0-500 mM using a modified Kunitz assay. Activity measured at 200 mM NaCl was set to 100%.

Simple to Optimize in Cell Media

M-SAN HQ is uniquely formulated to excel in physiological pH conditions, offering high performance at the commonly used cell media pH of 7.4.

While other nucleases often require more alkaline environments, M-SAN HQ stands out for its adaptability and effectiveness in cell media. It's the nuclease that truly aligns with your bioprocessing needs.

Properties & Ordering Information: See page 15



M-SAN HQ has excellent performance at typical cell media pH.
Activity was measured at 37°C in a 25 mM Tris-HCl or Bis-Tris buffer at various pH, 2.5 mM $MgCl_2$ and 150 mM NaCl using a modified Kunitz assay. Activity measured at pH 7.7 was set to 100%.

> When compared with non-salt-tolerant endonucleases (Benzonase® and DENARASE®), salt-tolerant endonucleases have better performance at the salt concentrations and pH naturally present in the cell culture supernatant, allowing its direct application without further processing. In addition to that, salt-tolerant endonucleases allowed the removal of chromatin, one of the most critical impurities and often overlooked in enveloped virus DSP.

- Mayer et al, Biotechnology Progress, First published: 27 March 2023, DOI:(10.1002/btpr.3342)

SAN HQ: The Ultimate Solution for DNA Clearance in Adenovirus & AAV Bioprocessing

Salt Active Nuclease High Quality (SAN HQ) and SAN HQ GMP are Bioprocessing Grade nucleases developed as the most efficient solution for removal of nucleic acids, at high salt conditions. This nonspecific endonuclease has peak activity at salt concentrations between 400 – 650 mM.

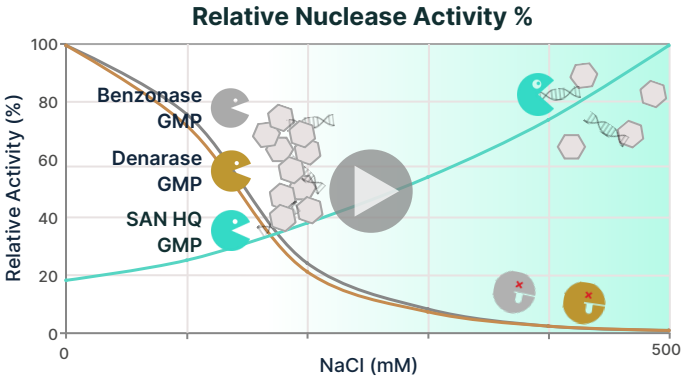
Open new horizons with high-salt-tolerant nucleases

Non-enveloped viruses like Adenoviruses and Adeno-Associated Viruses (AAV's) are inherently robust with two distinct advantages:

- they exhibit higher tolerance to additives like salt and detergents
- their production often involves the lysis of host cells, to harvest non-secreted vectors

For Adeno-Associated Viruses (AAVs), which are often harvested from crude cell lysate, the high salt tolerance of SAN HQ is particularly beneficial. Salt is typically added to such lysates to reduce viral aggregation, which also facilitates more effective nuclease action to digest residual DNA.

Optimized for high-salt environments, SAN HQ ensures unparalleled DNA removal without compromising the integrity of robust viral vectors.

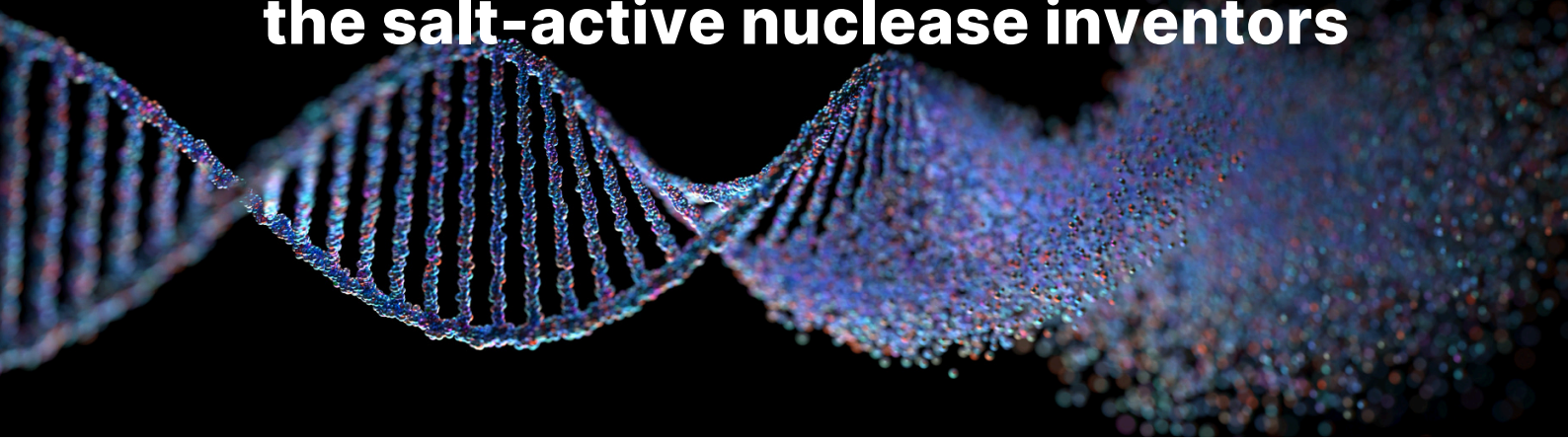


See the nucleases in action in this 30 sec. video

Now also in GMP Grade

SAN HQ GMP

Where GMP-grade meets the innovation of the salt-active nuclease inventors



Why Reducing Residual DNA is Vital

Residues from the manufacturing process must be below strict limits for products administered to humans, so it is imperative to understand the mechanisms for reducing and detecting these residues to ensure safety and compliance.

The Big Picture

Reducing residual DNA in lysate is not a procedural step; it's a regulatory must-do that directly impacts product quality and safety.

Zoom In

SAN HQ effectively degrades Chromatin DNA to sizes that can be fully trapped and removed during purification, ensuring compliance with strict regulatory criteria.

Why It Matters

Effective DNA clearance not only ensures AAV vector purity but also minimizes patient risk. It's not just about compliance; it's about advancing the quality of gene therapy.

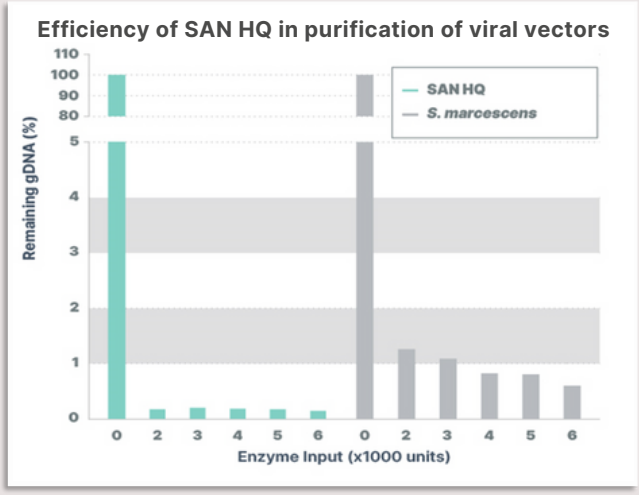
The Bottom Line

DNA clearance is non-negotiable for compliance, safety, and market success.

Key benefits of combining high salt and SAN HQ:

- **Regulatory Compliance**: SAN HQ facilitates digestion of residual DNA, aiding in compliance with FDA standards. **SAN HQ GMP offers an additional layer of regulatory assurance.**
- **Product Safety**: SAN HQ minimizes contaminating host cell residual DNA, enhancing the safety profile of gene therapy products.
- **Product Purity**: By reducing residual DNA, SAN HQ improves product purity.
- **Efficiency**: Streamlines downstream purification by reducing DNA-induced viscosity challenges.
- **Product Quality**: Ensures the final product primarily consists of therapeutic viral vectors for consistent, high-quality outcomes.
- **Yield:** Increases viral vector yield by reducing aggregates.

> Given its optimal activity in high salt conditions, SAN HQ improves downstream processes and reduces purification time without loss of vector yield and activity. SAN HQ's improved efficiency is serotype independent.
>
> - Evaluation by a viral vector core lab at a major academic center in mid-Atlantic region (US)



Using SAN HQ resulted in better clearance using less nuclease
Material was sampled both before and after endonuclease treatment (at 37°C for 1 hr) at optimum buffer conditions. Genomic DNA was quantified using the Quant-iT dsDNA High Sensitivity Assay Kit (Invitrogen) and normalized to pre-enzyme treatment samples.

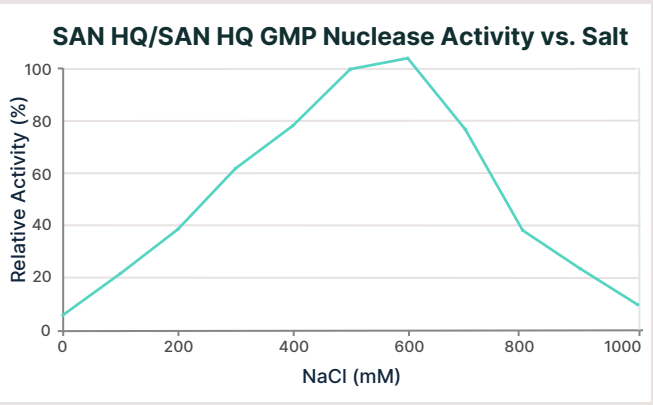
Optimized for Performance Where it Matters - Bioprocessing with SAN HQ and SAN HQ GMP

These Bioprocessing Grade nucleases are the most efficient solution for removal of both single and double stranded DNA and RNA, at high salt conditions.
These nonspecific endonucleases have peak activity at salt concentrations between 400 – 650 mM.

Engineered for optimum activity in these high salt environments ensuring unparalleled DNA removal without compromising the integrity of these robust viral vectors.

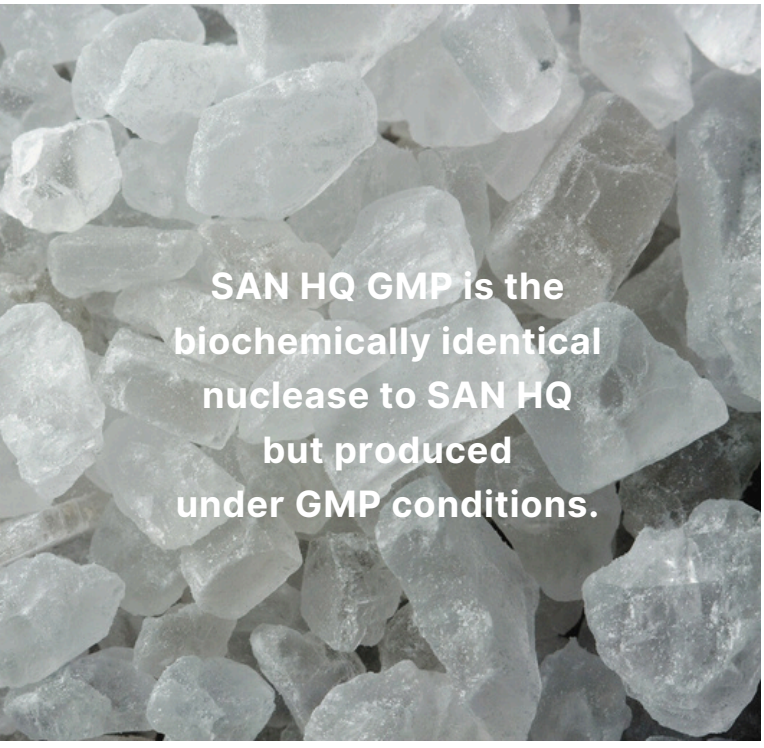
Applications:
Removal of nucleic acids during protein production, vaccine manufacturing and viral vector preparation.

Properties & Ordering Information: See page 15

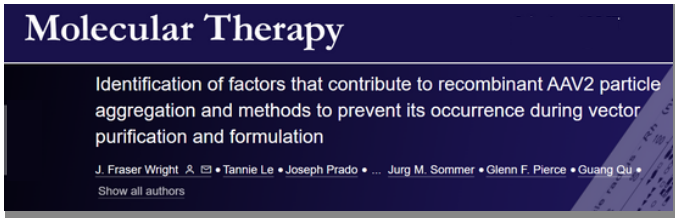


Peak activity in range that matters most for AAV's
SAN HQ GMP exhibits increasing enzymatic activity as salt concentrations rise, peaking at 600 mM for pH 8.5 at 37°C.
This is where typical salt concentration range is ideal for processing non-enveloped viral vectors like Adenoviruses and AAV"s.

I ♥ NaCl



Benzonase struggles with DNA clearance in high-salt conditions, as cited in Molecular Therapy.



> The conditions that we used for Benzonase digestion of crude cell lysates did not achieve the degree of removal of vector surface DNA required to prevent aggregation in subsequently purified vector. This may pertain specifically to our protocol, which required an elevated salt concentration to efficiently extract vector from cells, conditions not optimal for Benzonase activity.

M-SAN HQ

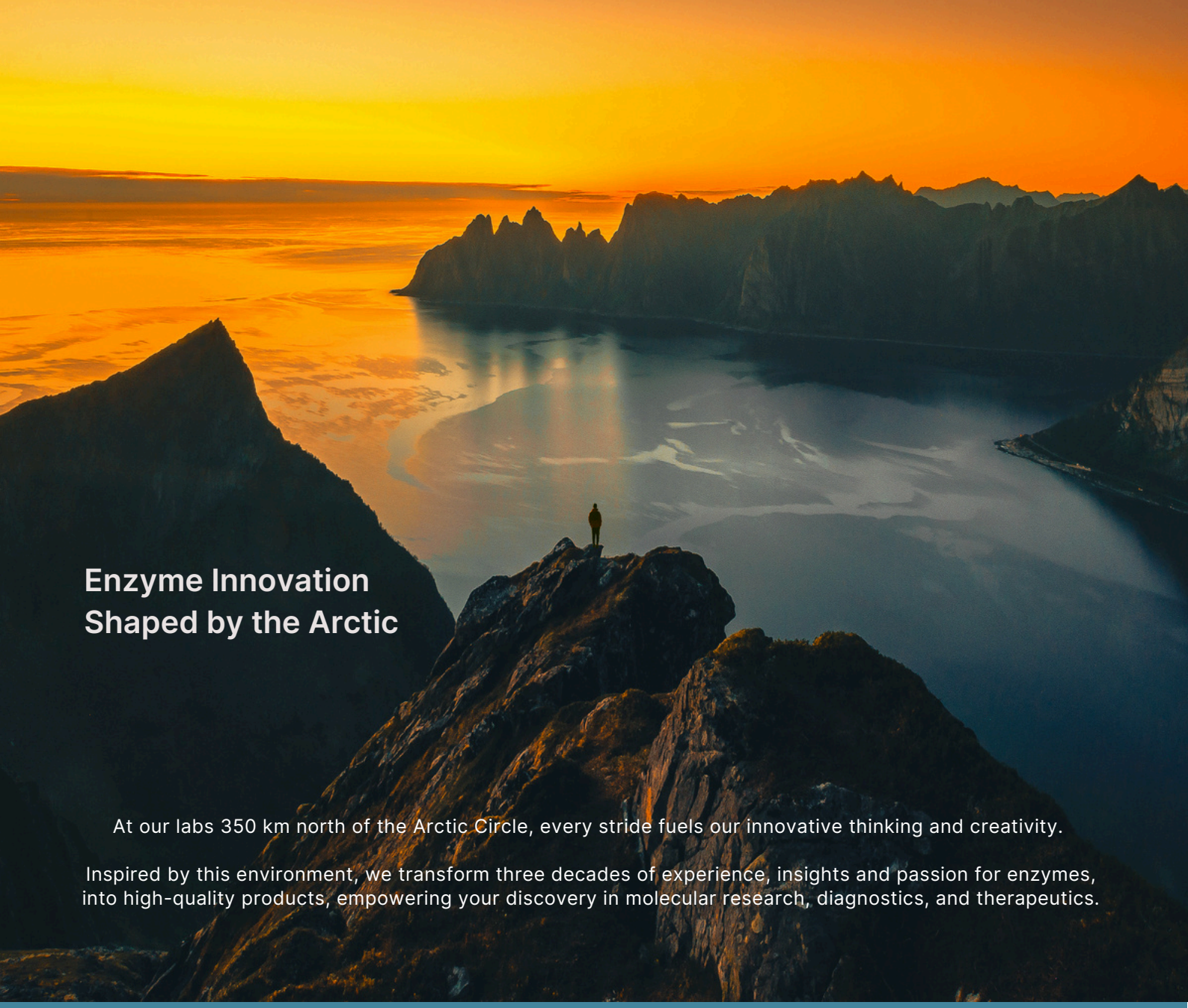
Source	Recombinantly produced in Pichia pastoris	**Specificity**	Nonspecific endonuclease cleaving single and double stranded DNA and RNA.
Molecular weight	24.5 kDa	**Working ranges**	• Temperature: 20 – 50°C, optimal: 36 – 50°C • Salt concentration (NaCl): 0 – 400 mM, optimal: 90 – 225 mM • Mg^{2+}: > 0.5 mM is required for activity, optimal: 4 - 15 mM • pH: 6.3 – 8.7, optimal: 7.2 - 8.7 Note: The working range is defined as above 10% activity and optimal range as above 80% activity.
Protein purity	≥ 99% by SDS-PAGE analysis		
Isoelectric point	8.62		
Unit definition	One unit is defined as the amount of enzyme that causes a ΔA260 = 1.0 in 30 minutes at 37°C in 25 mM Tris-HCl pH 7.6 (@25°C), 2.5 mM $MgCl_2$, 150 mM NaCl, and 50 µg/ml calf thymus DNA.	**Tolerance to typical buffer additives**	• Triton X-100: No reduction in activity (tested up to 15%) • Tween20: No reduction in activity (tested up to 10%) • DTT and other reducing agents may inactivate M-SAN HQ • Urea: Not recommended

SAN HQ / SAN HQ GMP

Source	Recombinantly produced in Pichia pastoris	**Specificity**	Nonspecific endonuclease cleaving single and double stranded DNA and RNA.
Molecular weight	24.8 kDa. Molecular Weight is theoretically calculated from the peptide sequence	**Working ranges**	• Temperature: 5 – 38°C, 4°C overnight, optimal: 32 - 38°C • Salt concentration (NaCl / KCl): 0 – 1000 mM, optimal: 400 – 700 mM • Mg^{2+}: >1 mM is required for activity, optimal: 6 - 50 mM • pH: 7.0 - 9.5, optimal: 8.0 - 8.8 Note: The working range is defined as above 10% activity and optimal range as above 80% activity.
Protein purity	≥ 98% by SDS-PAGE analysis		
Isoelectric point	9.55		
Unit definition	One unit is defined as the amount of enzyme that causes a ΔA260 = 1.0 in 30 minutes at 37°C in 25 mM Tris-HCl pH 8.5 (@25°C), 5 mM $MgCl_2$, 500 mM NaCl, and 50 µg/ml calf thymus DNA.	**Tolerance to typical buffer additives**	• Imidazole: 20% activity at 350 mM Imidazole • Glycerol: 20% activity at 35% Glycerol • Triton X-100: No reduction in activity (tested up to 15%) • Tween20: No reduction in activity (tested up to 10%) • SDS: Not recommended • Urea: Not recommended • Reducing agents (e.g. DTT, TCEP): will result in inactivation

Ordering Information

	REACH Compliant	Article no.	Pack size	Concentration
SAN HQ	Yes	70920-202	25 kU	25 - 30 U/µl
	Yes	70920-150	500 kU	≥ 250 U/µl
	Yes	70920-160	5 MU	≥ 250 U/µl
	Yes	70920-100	Custom	Custom
SAN HQ Triton Free	Yes	70921-202	25 kU	25 - 30 U/µl
	Yes	70921-150	500 kU	≥ 250 U/µl
	Yes	70921-160	5 MU	≥ 250 U/µl
	Yes	70921-100	Custom	Custom
SAN HQ GMP	Yes	70980-150	500 kU	400 – 600 U/µl
	Yes	70980-160	5 MU	400 – 600 U/µl
SAN HQ ELISA	Yes	70930-001	1 x 96 Well Plate	N/A
M-SAN HQ	Yes	70950-202	25 kU	25 - 30 U/µl
	Yes	70950-120	200 kU	≥ 250 U/µl
	Yes	70950-150	500 kU	≥ 250 U/µl
	Yes	70950-155	1 MU	≥ 250 U/µl
	Yes	70950-160	5 MU	≥ 250 U/µl
	Yes	70950-100	Custom	Custom
M-SAN HQ ELISA	Yes	70960-001	12 x 8 Strip Plate	N/A



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