ArcticZymes Technologies

Salt Active Nucleases

For Bioprocessing

www.arcticzymes.com

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Cell and gene therapies and viral vector-based vaccines are currently among the most promising therapeutic areas. In these therapies, engineered viruses are commonly used as vectors to deliver and insert genetic material into cells to treat or prevent disease. Among the most promising tools are vectors based on adenoviruses, adeno-associated viruses (AAVs) and lentiviruses. To drive clinical studies and commercialisation, the development of scalable, robust and high-yielding manufacturing methods for these vectors remains a key challenge for the industry.

Ideal for multiple bioprocessing and biomanufacturing workflows

SAN
High Quality

M-SAN
High Quality

SAN HQ and M-SAN HQ are especially developed to suit the high quality and regulatory requirements for use in bioprocessing workflows. Their biochemical properties make them ideal for multiple bioprocessing and biomanufacturing workflows.

Adapted to the challenge

Due to safety concerns, regulators have imposed limits on the amount and length of nucleic acid residues that can be present in the final dosages of therapeutics for human administration. The industry standard for removing residual DNA in bioprocessing workflows is to use nuclease treatment in the downstream process.

The conditions for the nuclease treatment vary across vectors and based on downstream considerations. Enveloped vectors such as lentiviruses, are fragile, and therefore typically kept at close to physiological conditions to prevent their integrity. This also applies during the nuclease treatment step.

Non-enveloped vectors such as adenoviruses and AAVs generally tolerate a wider range of buffer conditions. The viral capsids of these vectors are highly charged, and therefore prone to forming complexes with DNA and other impurities in the medium. This can lead to vector aggregation, and to co-purification of the vector and the impurities, ultimately reducing viral titer and purity.

High-salt buffers can be used to counteract electrostatic interactions between capsids and impurities, thereby reducing aggregation. An additional benefit of increasing the salt concentration is that it makes chromatin-DNA from the host-cell more accessible for digestion by similar mechanisms.

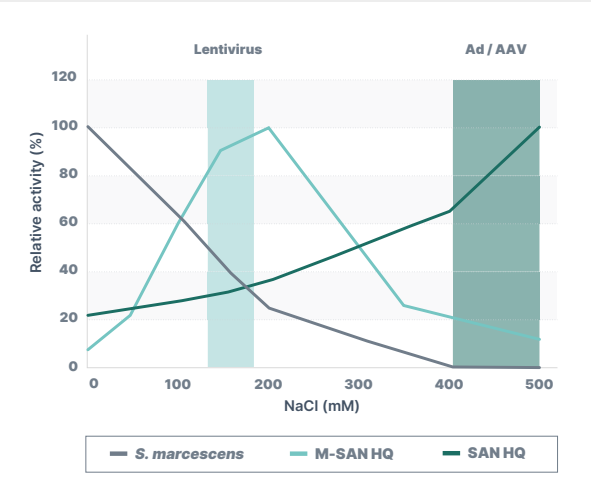


Fig 1. Optimal performance at downstream conditions

The salt active nucleases are adapted to conditions typically encountered in downstream processing of viral vectors.

Several commercially available endonucleases used in bioprocessing originate from *Serratia marcescens*. These nucleases do not perform optimally at the physiological conditions found in cell media, nor do they tolerate high salt concentrations. To compensate for the loss of activity, the workflow is sometimes adapted to the nuclease, for instance by introducing a desalting step, or by increasing the nuclease concentration.

ArcticZymes' salt active nucleases are tailor made for viral bioprocessing, which allows for a more streamlined, less expensive and more efficient downstream process. Due to their high isoelectric points, they can be readily removed after use by capture on cationic IEX columns.

SAN High Quality

Bioprocessing grade

Superior actvity at high salt conditions

High purity (≥ 98%)

Active at low temperatures

Compatible with SAN HQ ELISA

SAN High Quality is the ultimate solution for efficient removal of nucleic acids in high-salt manufacturing and bioprocessing workflows. This nonspecific endonuclease has optimum activity at salt concentrations between 400 – 650 mM.

SAN High Quality has optimal activity at high-salt

Salt is an important component in many purification processes. The presence of salt can reduce aggregation, increase target solubility, and improve target yield. High-salt enables contaminating DNA to dissociate from associated proteins and become available for degradation. SAN High Quality is highly compatible with the use of high-salt conditions, which in many cases allows for significant improvements in efficiency and yield.

Application: DNA removal in high-salt lysates

SAN High Quality is ideally suited for DNA removal in mammalian cell lysates and supernatants supplemented with salt (Fig 2). In this case, HEK 293 cells were grown in DMEM for 48 hrs before lysis and nuclease treatment at high salt (50 U/ml nuclease, 500 mM NaCl, 5 mM Mg^{2+}). Remaining DNA after 1 hr incubation at 37°C was quantified using Quant-iT™ PicoGreen™ dsDNA Assay Kit.

Ideal for high-salt lysates

By taking advantage of the superb activity of SAN HQ in combination with the increased availability of DNA at high-salt concentrations, highly effective DNA clearance can be achieved early in the downstream process. In this case a more than 20-fold reduction in residual DNA was achieved. The other tested nucleases showed inferior performance relative to SAN HQ at the tested conditions.

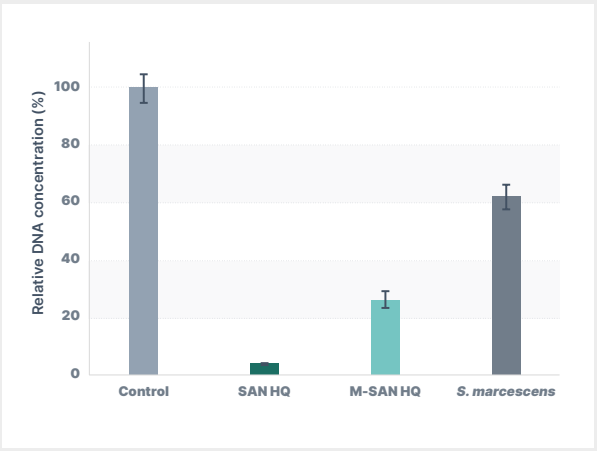


Fig 2. SAN HQ is ideal for treatment of high-salt lysates and supernatants

SAN HQ outperforms other nucleases in removal of host-cell DNA from HEK 293 cells in high-salt lysis buffers.

Properties

Source	Recombinantly produced in *Pichia pastoris*
Molecular weight	The protein is glycosylated. Protein size without glycosylation is 26 kDa.
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.

Specificity	Nonspecific endonuclease cleaving single and double stranded DNA and RNA.
Working ranges	• Temperature: 5 – 40°C, optimal: ~35°C • Salt concentration (NaCl / KCl): 150 – 900 mM, optimal: 400 – 650 mM • Mg^{2+}: >1 mM is required for activity, optimal: 5 - 50 mM • pH: 7.3 – 10.0, optimal: 8.2 - 9.2 Note: The working range is defined as ~20% of activity and optimal range is ~80% of activity
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%) • SDS: Not recommended • Urea: Not recommended • Reducing agents (e.g. DTT, TCEP): will result in inactivation

M-SAN High Quality

Bioprocessing grade

M-SAN HQ is the recent addition to our salt-active nuclease portfolio. M-SAN HQ has been developed for removal of nucleic acids at the near-physiological conditions used in many bioprocessing and biomanufacturing workflows, and outperforms other commercially available nucleases at these conditions.

Can be directly used in medium without buffer adjustments

This novel, nonspecific endonuclease is active over a broad pH range and displays optimum activity at salt concentrations between 125 – 250 mM. Due to the excellent performance at physiological conditions, M-SAN HQ can be used directly in the cell medium or the harvested supernatant, without buffer adjustments. This makes M-SAN HQ suitable for manufacturing of fragile vectors such as lentiviruses.

Excellent performance at physiological conditions

High purity (≥ 99%)

Compatible with M-SAN HQ ELISA

Application: DNA removal directly in cell medium

M-SAN High Quality has optimal activity around physiological conditions, which makes it ideal for DNA removal directly in mammalian cell media (Fig 3). In this case, HEK 293 cells were grown in DMEM for 48 hrs before nuclease treatment directly in medium (75 U/ml nuclease). The medium was supplemented with Mg^{2+} to 5 mM. Remaining DNA after 1 hr incubation at 37°C was quantified using Quant-iT™ PicoGreen™ dsDNA Assay Kit.

Optimal performance at physiological conditions

The high activity of M-SAN HQ at standard cell medium conditions leads to improved DNA clearance compared to other commonly used nucleases. In this case, a 5-fold reduction in residual DNA was achieved.

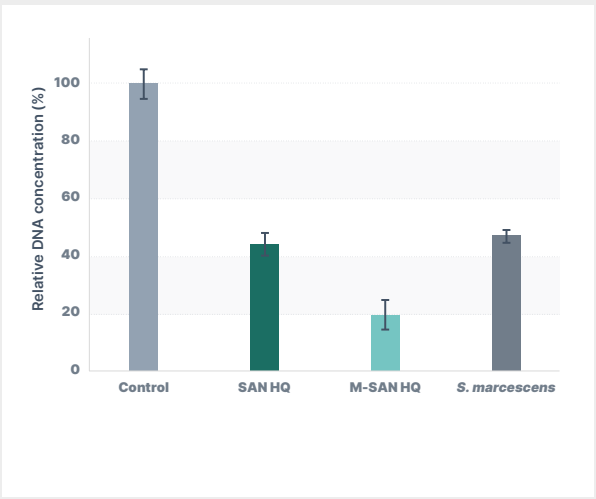


Fig 3 M-SAN HQ is effective when used directly in cell media.

M-SAN HQ outperforms other nucleases in removal of host-cell DNA from HEK 293 cells directly in cell media.

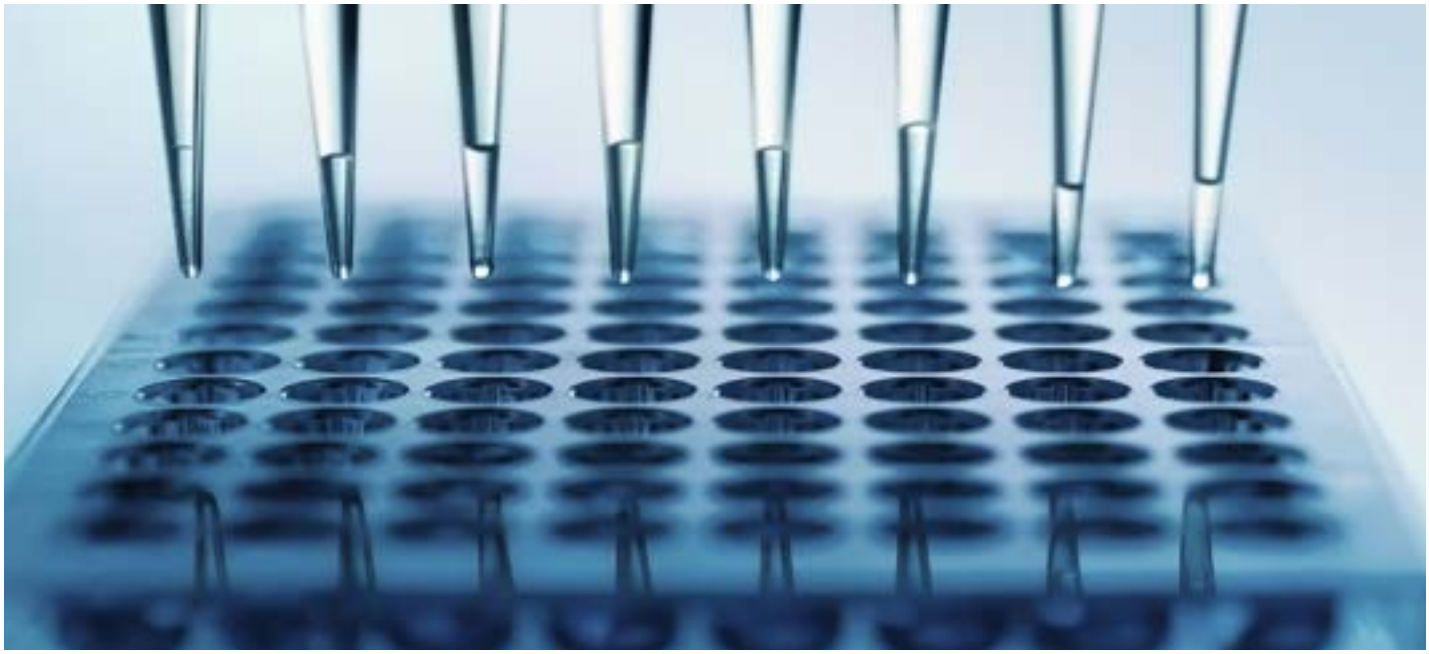
Properties

Source	Recombinantly produced in *Pichia pastoris*
Molecular weight	24.5 kDa
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.

Specificity	Nonspecific endonuclease cleaving single and double stranded DNA and RNA.
Working ranges	• Temperature: 25 – 50°C • Salt concentration (NaCl): 50 – 400 mM, optimal: 125 – 250 mM • Mg^{2+}: > 0.5 mM is required for activity, optimal: 4 - 15 mM • pH: 6.5 – 9.5, optimal: 7.2 - 8.7 Note: The working range is defined as ~20% of activity and optimal range is ~80% of activity
Tolerance to typical buffer additives	• DTT and other reducing agents may inactivate M-SAN HQ • Urea: Not recommended • EDTA: Not recommended

No license required

At ArcticZymes, we pride ourselves on always offering seamless accessibility to our high-quality products. Produced under ISO 13485, our enzymes are sold under a “no license required” policy to ensure that our customers are not restricted by legal burdens, now or with their future use. In addition, we offer our nucleases in a flexible format and are readily available to discuss your custom needs.



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

Your OEM Partner to deliver novel solutions for genomics and proteomics

Quality

Our bioprocessing grade nucleases are manufactured according to requirements in ISO 13485. In addition, relevant requirements from cGMP have been implemented. The nucleases are manufactured using only non-animal origin raw materials to minimize the risk of contamination with adventitious agents. The final product is sterile filtered (0.22 µm), and release tests include both bioburden (TYMC/TAMC) and endotoxin assays according to USP-harmonised European Pharmacopeia methods.

By being the original manufacturer of SAN HQ and M-SAN HQ, 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.

We believe by this approach ArcticZymes Technologies offers an attractive balance between quality and cost for their customers.

ELISA Kits

ArcticZymes offers ELISA kits to confirm the removal of SAN High Quality and M-SAN High Quality in bioprocessing and biomanufacturing applications.

Additional information

For more information, please check our website www.arcticzymes.com.

Cutting-edge enzymes from Norway

ArcticZymes Technologies has a long history dating back to the late 1980s. Based in Tromsø, Northern Norway, we use access to the marine Arctic to identify new cold-adapted enzymes for use in molecular research, *in vitro* diagnostics and therapeutics. We focus on strong and reliable relationships with our business partners and commercial innovators around the world. Therefore, we are constantly striving to work at the highest level and not only meet but exceed the expectations of our partners.

In the service of science

The knowledge of the important role our enzymes play in research, diagnostics and therapeutics drives us every day. Our team of highly motivated and experienced scientists is constantly developing further innovations in order to expand our portfolio of novel and high-quality solutions.

A partner you can trust

Security of supply

With us you are always on the safe side when it comes to the timely delivery of high-quality enzymes. We strive for a reliable and uninterrupted supply of whatever enzyme technology you need.

Partnership approach

Our focus is on cooperative B2B partnerships which means that we put our customers' needs at the center of what we do. We strive to provide innovative solutions in order to help them to succeed in whatever they do.

Unique enzyme features

Enzymes play a decisive role in molecular research, *in vitro* diagnostics and therapeutics. This makes it all the more important that they have a consistently high quality. Our novel enzymes are reproducible and have unique properties that make them particularly robust.

Unique access

Direct access to unique and diverse resources for bioprospecting allows us to continuously develop novel enzyme technologies with unique features and make them available to our partners.

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