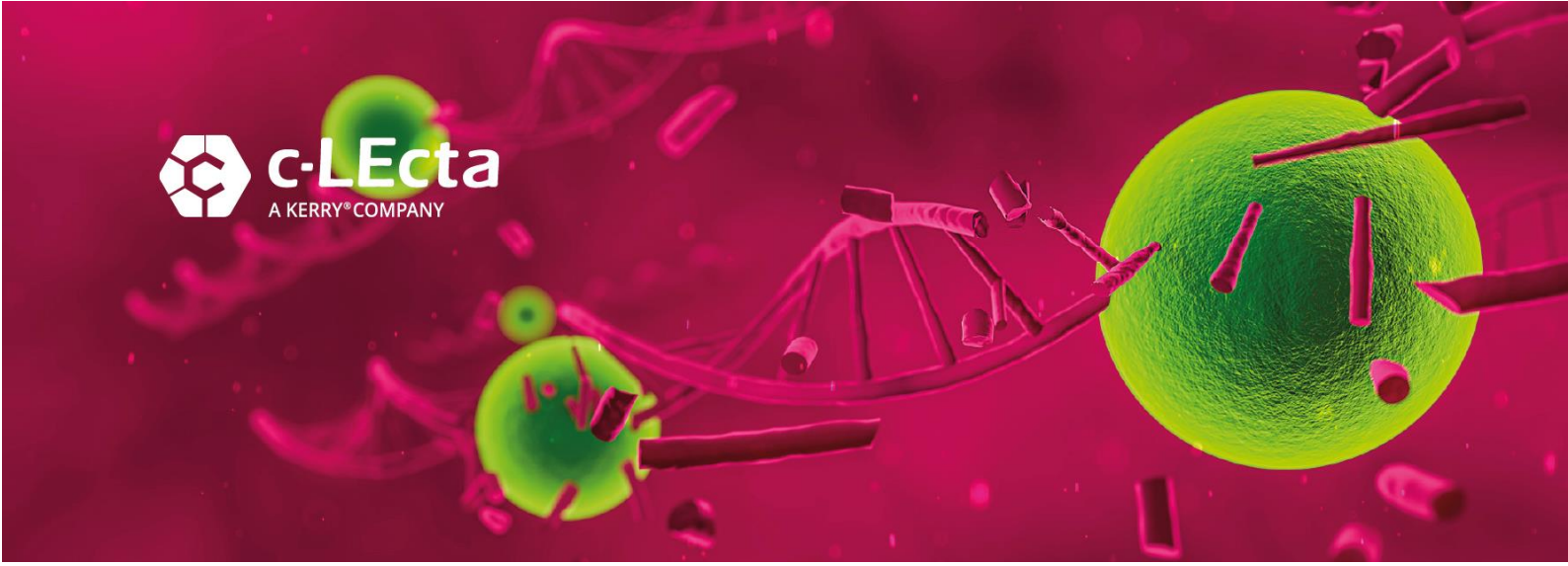

PRODUCT INFORMATION

DENARASE® High Salt

Recombinant *Serratia marcescens* endonuclease, genetically engineered for higher salt tolerance, liquid.

DENARASE High Salt is a recombinant *Serratia marcescens* endonuclease that is genetically engineered to enable use at elevated salt conditions without loss of activity. Similar to the wild type, the enzyme efficiently cleaves all forms of DNA and RNA into smaller nucleotides. DENARASE High Salt shows peak performance over a wide range of bioprocessing-relevant salt concentrations and pH conditions. This flexibility makes DENARASE High Salt the perfect solution for the removal of process-related nucleic acids in diverse biomanufacturing processes that require the presence of high monovalent cation levels.

1 Typical Applications

DENARASE High Salt enables optimal and cost-effective removal of nucleic acids in biomanufacturing processes that benefit from higher salt concentrations. The enzyme is especially suitable for the use at bioprocessing-relevant 200-500 mM salt and pH 7.4 – pH 8. It is designed to improve the efficiency of various applications:

- Viral Vectors for Cell & Gene Therapies
- Viral Vaccines
- Viscosity reduction in Lysates
- Sample preparation in Electrophoresis and Chromatography.

2 Compliance

DENARASE High Salt is intended for use in research and development (R&D) applications and process development of biologicals such as viral vectors and vaccines. The R&D-grade DENARASE High Salt is produced under ISO 9001 standard, without the use of antibiotics, Triton X-100 and raw materials of animal origin.

The launch of the DENARASE High Salt GMP-grade for use in commercial manufacturing processes of biologicals is scheduled for Q2 2025. The manufacturing process of the DENARASE High Salt GMP-grade complies with the same regulations and standards as our well-known wild-type GMP-grade DENARASE products. This includes manufacturing under EU GMP conditions acc. to EU GMP regulations and distribution compliant with EXCiPACT and ANSI/NSF 363 Standard and thus also meet the requirements for Good Manufacturing and Distribution Practices (GMP/GDP) for pharmaceutical excipients. From a technical performance perspective, DENARASE High Salt R&D- and GMP-grade are equal.

3 Removal of DENARASE High Salt

Common purification techniques like chromatography and tangential flow filtration can be used to remove DENARASE High Salt from process intermediates.

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4 ELISA Kit

The amino acid sequence of the DENARASE High Salt differs from the wild-type enzyme in only a few amino acids. For this reason, the DENARASE ELISA Kit for analysis of endonucleases from *Serratia marcescens* can also be used for the detection and quantification of DENARASE High Salt. As the kit only contains a standard solution of the wild-type enzyme, it is necessary to multiply ELISA kit readings of DENARASE High Salt by a factor of 1.46 to obtain accurate protein concentrations. Alternatively, a DENARASE High Salt standard solution can be generated.

5 Operating conditions

Like the wild-type *S. marcescens* endonuclease, DENARASE High Salt is a robust enzyme that shows DNA clearance activity under various conditions. Engineered to perform under a broad range of monovalent cation concentrations (**Fig. 1** & **Fig. 2**), the enzyme's activity is influenced by further process parameters including temperature, pH and presence of cofactors and inhibitory compounds. To determine optimal operating conditions, DENARASE High Salt activity was measured under standard conditions following a protocol similar, but not identical to the c-LEcta release test protocol for DENARASE High Salt (detailed in the DENARASE High Salt Validation Guide, Section 3.1). Therefore, enzyme activities were normalized as indicated.

5.1 Salt concentration

The presence of monovalent cations dose-dependently inhibits the activity of the wild-type DENARASE enzyme. DENARASE High Salt exhibits robust activity over a broad spectrum of sodium chloride (NaCl) concentrations (**Fig. 1**). The activity of the salt-adapted enzyme increases with intermediate NaCl concentrations at pH 8, representing typical release test conditions of endonucleases. Additionally, DENARASE High Salt shows high activity across 0 – 500 mM NaCl at bioprocessing-relevant pH 7.4. Based on these salt profiles we recommend using DENARASE High Salt at salt concentrations above 200 mM and the wild-type DENARASE for monovalent cation concentrations below 200 mM.

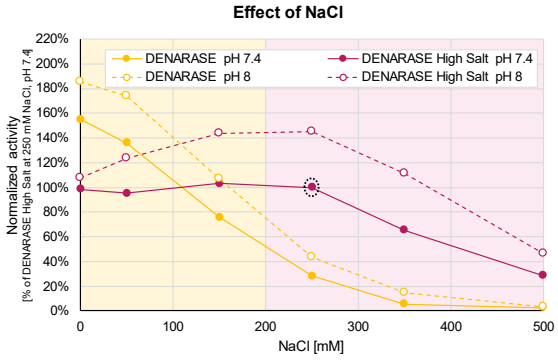

Fig. 1 The effect of increasing NaCl concentrations on the enzyme activity of the wild-type *S. marcescens* endonuclease (DENARASE) and DENARASE High Salt. The volumetric activities at 15 mM $MgCl_2$ and pH 7.4 as well as pH 8 were measured in U/ml. After normalization to the activities as given on the CoAs of the respective enzyme batches, enzyme activities were normalized to DENARASE High Salt activity at 250 mM NaCl and pH 7.4 (indicated by dotted circle). The yellow and pink hatched areas mark the recommended salt range for the use of DENARASE and DENARASE High Salt, respectively.

Compared to SAN HQ (Salt Active Nuclease High Quality, ArcticZymes), the DNA clearance activity of DENARASE High Salt is higher over a broader salt concentration range (**Fig. 2**). Furthermore, DENARASE High Salt shows high DNA clearance activity at bioprocessing-relevant low pH levels (pH 7.4, **Fig. 2** left). The activity can further be enhanced by increasing the presence of magnesium (see also Section 5.2.).

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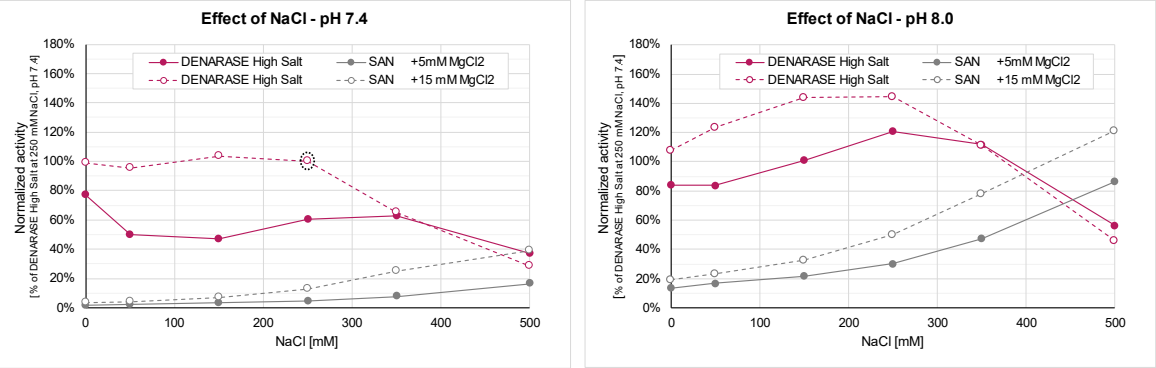

Fig. 2 The effect of increasing NaCl concentrations on the enzyme activity of DENARASE High Salt and Salt Active Nuclease High Quality (SAN, ArcticZymes). The volumetric activities at pH 7.4 (left) and pH 8 (right) were measured in U/ml. After normalization to the activities as given on the CoAs of the respective enzyme batches, enzyme activities were normalized to DENARASE High Salt activity at 250 mM NaCl, 15 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

5.2 Magnesium

As for many other enzymes, magnesium (Mg^{2+}) is an essential co-factor and a prerequisite for the nucleic acid clearance activity of the *S. marcescens* wild-type enzyme. To determine the influence of magnesium on the performance of DENARASE High Salt, the enzyme activity was measured under standard conditions in presence of 0 – 100 mM $MgCl_2$. Requiring the presence of minimal magnesium levels for basal activity, DENARASE High Salt shows activity at the standard condition of 5 mM $MgCl_2$ and pH 7.4 (**Fig. 3**; indicated by dotted circle). Raising the magnesium levels up to 25 mM and increasing the pH value to 8.0 increases the endonuclease activity at all tested salt concentrations (**Fig. 3** & **Fig. 4**). However, excess of $MgCl_2$ will reduce DENARASE High Salt activity.

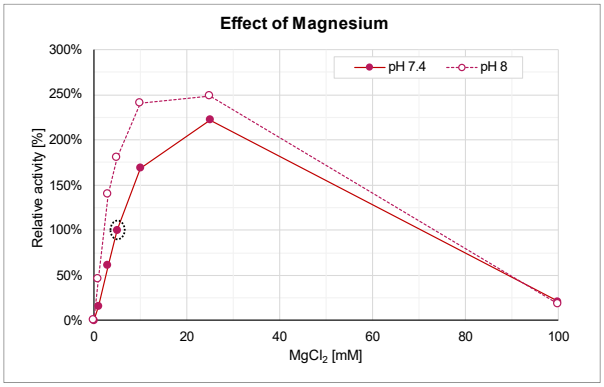

Fig. 3 Effect of low to high $MgCl_2$ concentrations on enzyme activity of DENARASE High Salt at 250 mM NaCl. The volumetric activities at pH 7.4 and pH 8 were measured in U/ml and normalized to activity at 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

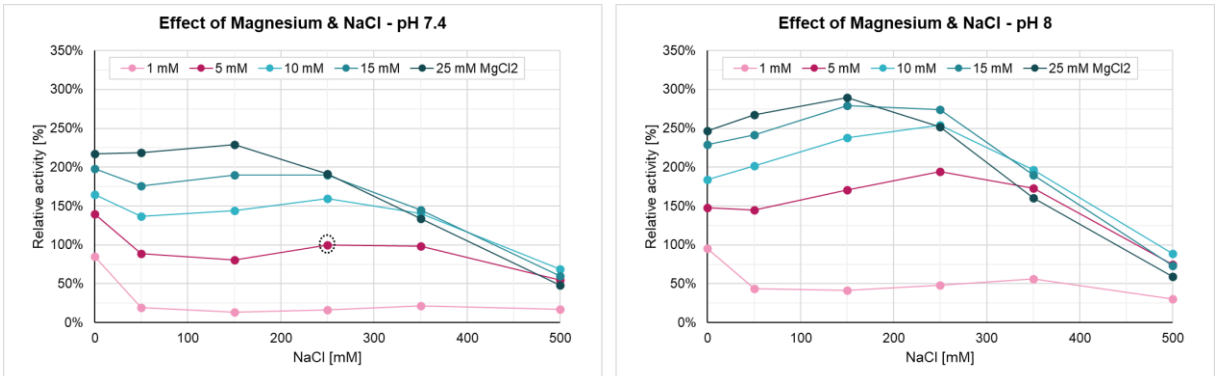

Fig. 4 Effect of low to intermediate $MgCl_2$ levels in combination with 0-500 mM NaCl on enzyme activity of DENARASE High Salt. The volumetric activities at pH 7.4 (left) and pH 8 (right) were measured in U/ml and normalized to activity at 250 mM NaCl, 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

DENARASE High Salt activity has been measured in frequently applied buffer systems such as Tris-HCl and phosphate buffers (**Fig. 5**). For all tested buffers the DENARASE High Salt activity was enhanced by increasing $MgCl_2$ concentrations, which thus can be a solution to circumvent the inhibitory effects of high phosphate concentrations.

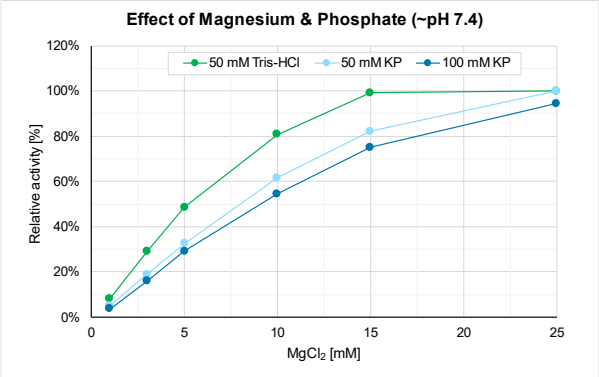

Fig. 5 Effect of $MgCl_2$ on DENARASE High Salt activity in Tris-HCl and potassium phosphate (KP) buffers. Volumetric activities at 250 mM NaCl were measured in U/ml and normalized to maximal enzyme activities.

5.3 Temperature & pH

To determine the optimal reaction temperature and pH value, DENARASE High Salt activity was measured under standard conditions (250 mM NaCl, 5 mM Mg^{2+}) at different temperatures and in several buffers at different pH values. The optimal reaction conditions for DENARASE High Salt are 37 °C and pH 7.4 – 9.0 (see **Fig. 6** & **Fig. 7**). Similar to other process parameters, high $MgCl_2$ levels enhance the activity of DENARASE High Salt at all temperatures and pH levels tested when compared to the standard magnesium levels.

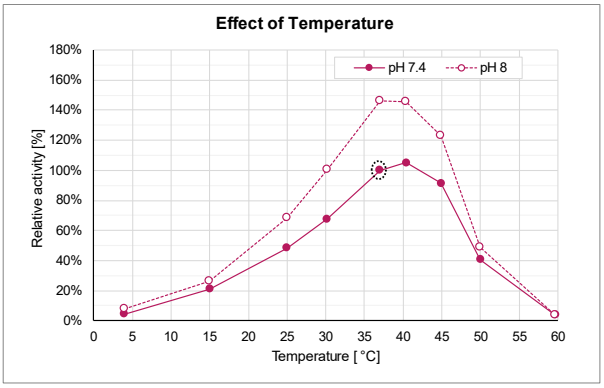

Fig. 6 Effect of temperature on DENARASE High Salt activity. Enzyme activity at different temperatures at pH 7.4 and 8.0 were normalized to activity at 37 °C, 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

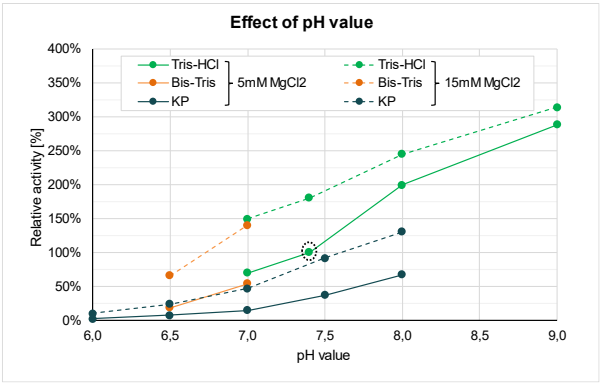

Fig. 7 Effect of pH on DENARASE High Salt activity. Enzyme activities in Tris-HCl, Bis-Tris and potassium phosphate (KP) buffer at different pH levels in the presence of 5 mM and 15 mM $MgCl_2$ were normalized to DENARASE High Salt activity at 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

6 Stability & Storage Conditions

When stored properly (-20 °C ± 5 °C), the enzyme is stable for at least 12 months from the date of product release.
Note: It is not recommended to store the product at -70 °C or below, since freezing the product will cause loss of activity. The long-term stability of DENARASE High Salt is currently under investigation.

7 Packaging Information

DENARASE High Salt is filled in non-pyrogenic, USP Class VI compliant vials. The product vials are shipped under qualified cooled conditions. Shipping temperature may differ from the storage temperature without affecting product quality. All DENARASE High Salt products will be delivered by c-LEcta in a sealed secondary packaging with tamper-evident seals.

8 Enzyme Characteristics

The enzyme catalyzes the hydrolysis of phosphodiesters of all forms of DNA and RNA, including single-stranded, double-stranded, linear, circular, or supercoiled forms into smaller nucleotides.

Molecular weight (calculated)	27 kDa (per monomer)
pH optimum	pH 7.4 - 9.0
Temperature optimum	37 °C
Isoelectric point (pI, calculated)	7.83[1]
Cofactor	Mg^{2+}

[1]Calculated using Clone Manager 11 Professional Edition (Sci Ed Software); reflects calculation acc. to Lehninger

9 Product Specification

Recombinant *Serratia marcescens* endonuclease, genetically engineered for higher salt tolerance, produced by microbial fermentation with *Bacillus* sp. The production strain employed in the manufacturing of the product is a Genetically Modified Organism (GMO) of safety level S1.
The enzyme is supplied as liquid and formulated in 20 mM Tris-HCl pH 7.4 ± 0.2, 250 mM NaCl, 5 mM $MgCl_2$, 50 % glycerol (v/v).
Produced under ISO 9001 standard.

Parameter	Method	Specification
Appearance	Visual	Clear, transparent solution
Activity	Photometric[1]	> 250 U/µl
Purity	Protein purity determined by SDS-PAGE and silver staining	≥ 99 %
Specific Activity	Activity per protein content determined photometrically at 280 nm with a molar extinction coefficient of 44,600 L x mol^{-1} x cm^{-1}	≥ 4 x 10^5 U/mg
Endotoxin level	LAL-Test acc. to Ph. Eur. 2.6.14, Method C	< 0.25 EU/kU
Total microbial count	TAMC/TYMC acc. to Ph. Eur. 2.6.12	Aerobic bacteria: < 5 cfu/200 µl Yeast/moulds: < 5 cfu/200 µl

[1] Unit-Definition: One unit (U) will digest salmon sperm DNA to acid-soluble oligonucleotides equivalent to a ΔA260nm of 1.0 in 30 min at pH 8.0 at 37 °C.

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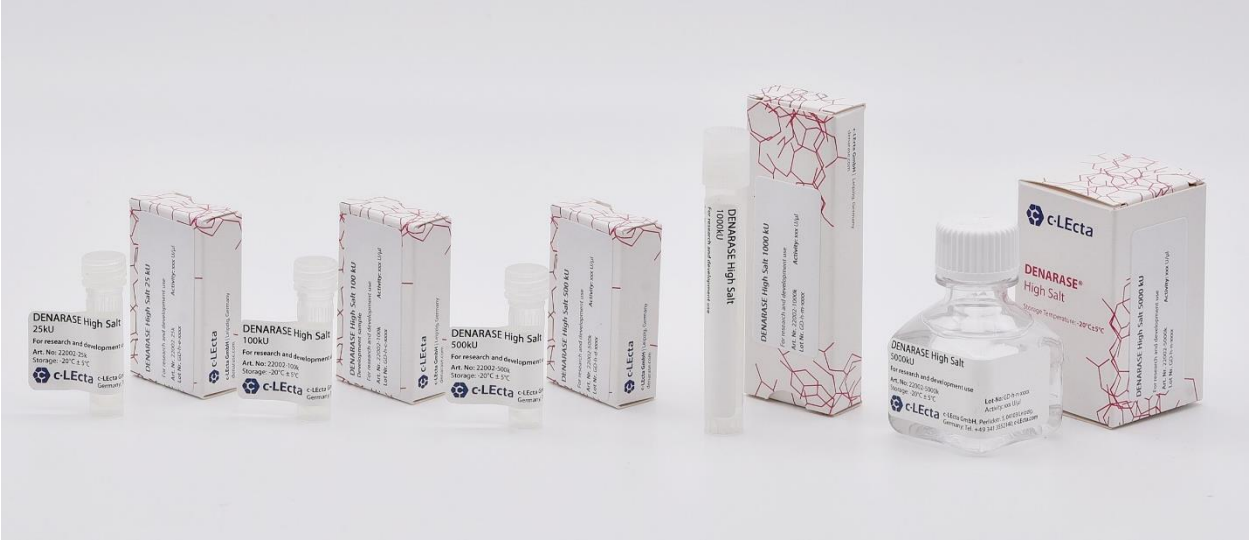
10 Sales and Contact

Products for use in research and development

Product	Art. No	Size	Activity	
DENARASE High Salt 25 kU	22002-25k	25 kU	> 250 U/µL	Produced under ISO 9001 standard
DENARASE High Salt 100 kU	22002-100k	100 kU	> 250 U/µL	Produced under ISO 9001 standard
DENARASE High Salt 500 kU	22002-500k	500 kU	> 250 U/µL	Produced under ISO 9001 standard
DENARASE High Salt 1000 kU	22002-1000k	1 MU	> 250 U/µL	Produced under ISO 9001 standard
DENARASE High Salt 5000 kU	22002-5000k	5 MU	> 250 U/µL	Produced under ISO 9001 standard



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