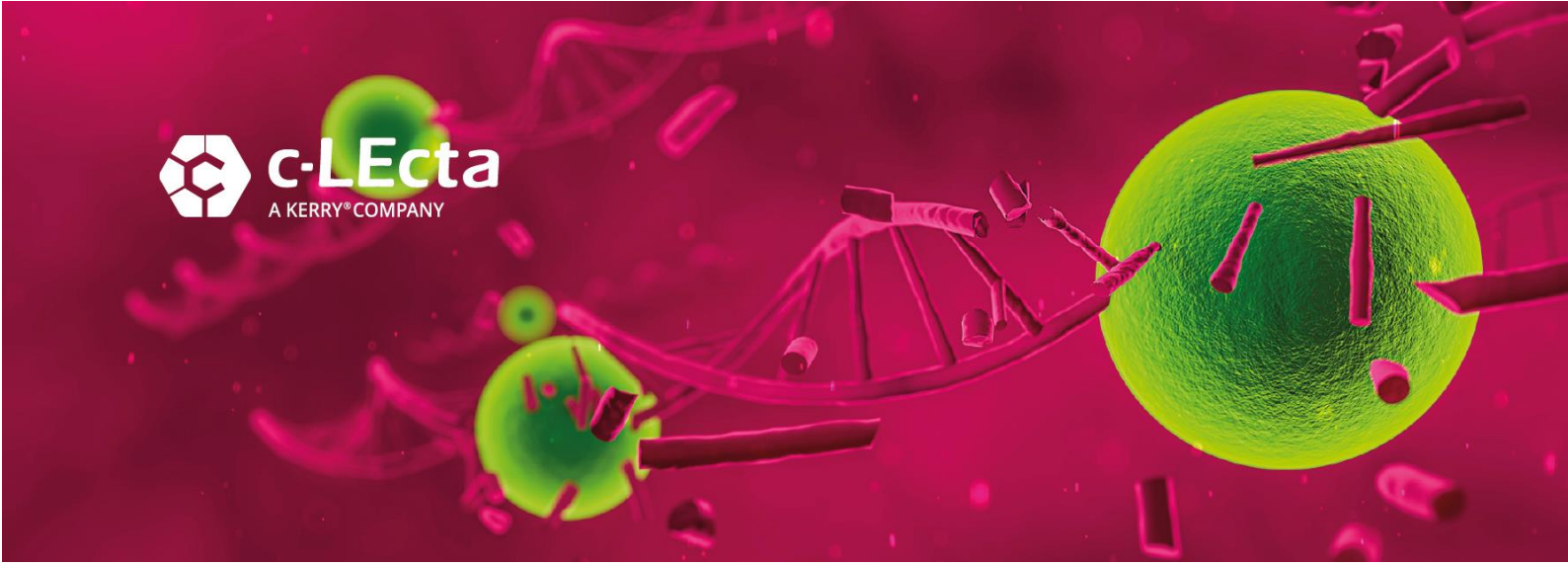

PRODUCT INFORMATION

DENARASE® High Salt

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

DENARASE High Salt is a recombinant *Serratia marcescens* endonuclease, genetically engineered to maintain activity at elevated salt conditions. Like the wild type, the enzyme efficiently cleaves all forms of DNA and RNA into smaller nucleotides. DENARASE High Salt is active across a wide range of bioprocessing-relevant salt concentrations and pH conditions. This improved flexibility makes DENARASE High Salt the ideal 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. For example, salt concentrations between 200-400 mM have been shown to improve the solubility of viral vectors (e.g., AAV) and DNA, reducing the formation of aggregates. Additionally, the increased solubility of DNA at elevated salt concentrations enhances its accessibility for degradation, leading to improved yield as well as purity and thus quality of viral vector-based drug products. DENARASE High Salt can be used at 0-500 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 available in two different quality grades:

The manufacturing process of the DENARASE High Salt GMP-grade complies with the same stringent regulations and standards as our well-known wild-type GMP-grade DENARASE products. Intended for the use in GMP manufacturing, this quality grade is produced under GMP conditions in accordance with EU GMP regulations, without the use of antibiotics, materials with TSE/BSE risk, or raw materials of animal origin. Additionally, the manufacturing and distribution of DENARASE High Salt by c-LEcta comply with the EXCiPACT® and ANSI/NSF 363 standards, ensuring adherence to Good Manufacturing and Distribution Practices (GMP/GDP) for pharmaceutical excipients.

In addition to DENARASE High Salt GMP-grade, DENARASE High Salt is also available in an R&D-grade, intended for the use in research and development (R&D). This quality grade is produced in compliance with the ISO 9001 standard, which has less stringent requirements for documentation, storage and distribution. It is ideal for R&D stages where quick and easy access to raw materials is crucial.
From a technical performance perspective, both the R&D- and GMP-grade of DENARASE High Salt are equivalent.

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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.

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 other 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 c-LEcta performance test* protocol which is 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).

5.1 Salt Concentration

The presence of monovalent cations such as Na^+ and K^+ dose-dependently inhibits the activity of the wild-type DENARASE enzyme. DENARASE High Salt exhibits high activity across 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 150 mM and the wild-type DENARASE for NaCl concentrations below 150 mM for optimal process efficiency.

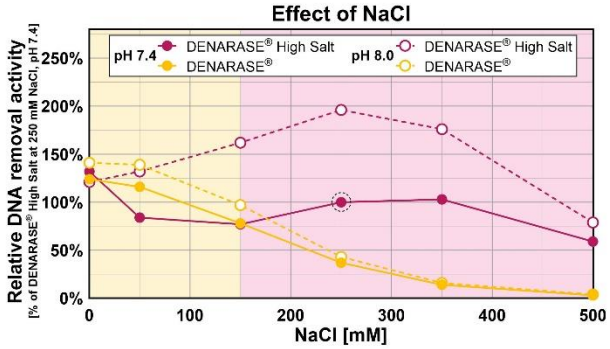

Fig. 1 The effect of increasing NaCl concentrations on the DNA removal activity of the wild-type *S. marcescens* endonuclease (DENARASE) and DENARASE High Salt was tested at 5 mM $MgCl_2$. and pH 7.4 as well as pH 8. Measured DNA removal activities [$Unit_{Test}$/µL] were first normalized to the applied nuclease units as specified in the respective CoAs [$Unit_{CoA}$/µL] and then 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.

* c-LEcta performance test: Nuclease products were tested at different NaCl concentrations in reactions conducted for 60 min at 30°C in 50 mM Tris-HCl (pH 7.4 and/or pH 8.0), 5 mM $MgCl_2$ and/or 15 mM $MgCl_2$, 0.8 mg/ml salmon sperm DNA, and 0.1 mg/mL bovine serum albumin. Reactions were stopped with perchloric acid, incubated on ice for 30 min, centrifuged, and the absorbance of the supernatants was measured at 260 nm and corrected against blanks without nuclease (ΔA_{260}). One test Unit ($Unit_{Test}$) of enzyme activity was defined as the amount of enzyme that produces a change in absorbance at 260 nm of 1.0 in the time of 30 minutes.

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Compared to other commercially available salt-active endonucleases (Salt-E1 – 4), DENARASE High Salt exhibits higher DNA removal activity across a wide range of salt concentrations, pH levels, and magnesium conditions (**Fig. 2**). It demonstrates high DNA clearance activity at bioprocessing-relevant low pH, especially with optimized magnesium supplementation (pH 7.4, **Fig. 2** bottom left). Overall, DENARASE High Salt activity can be further enhanced by increasing the presence of magnesium (see also Section 5.2).

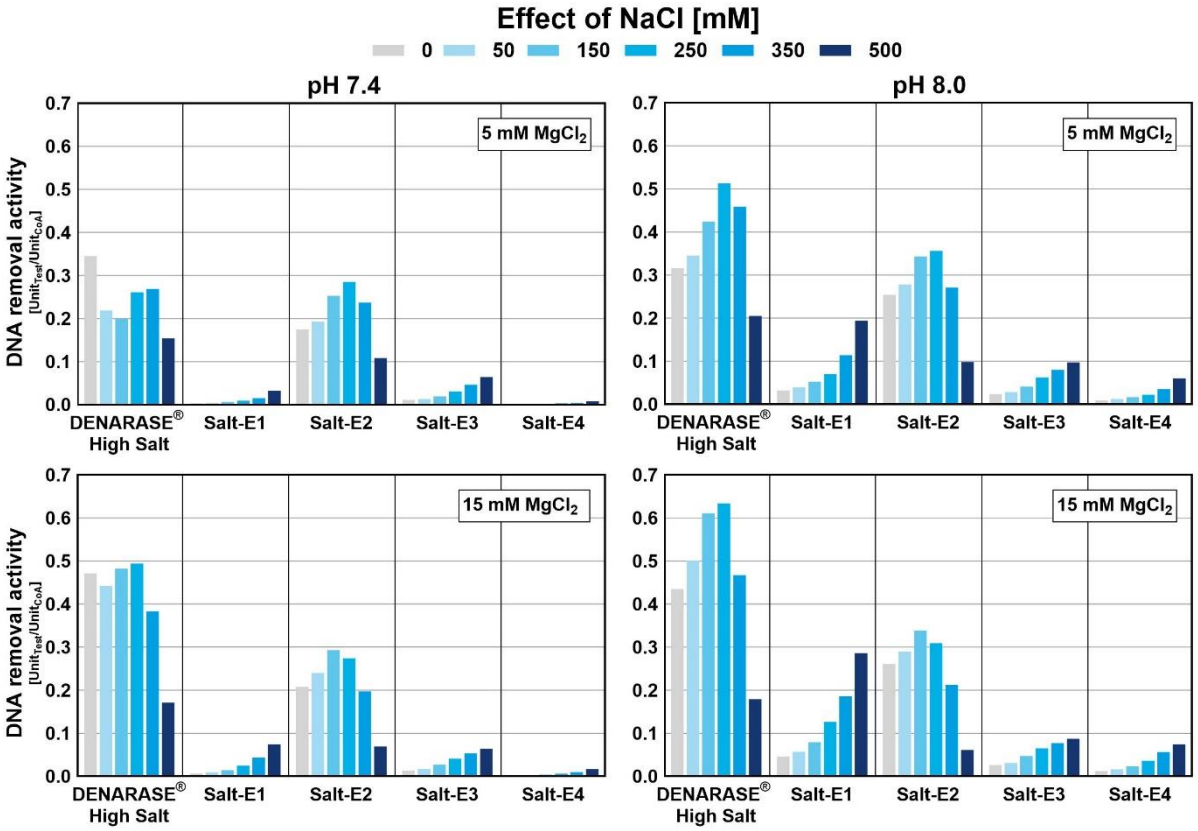

Fig. 2 The effect of increasing NaCl concentrations on the enzyme activity of DENARASE High Salt and other commercial salt-active endonucleases (Salt-E1 – 4). DNA removal activities were tested at NaCl concentrations of 0, 50, 150, 250, 350, and 500 mM (pH 7.4 and pH 8.0) in presence of 5 mM $MgCl_2$ (top) and 15 mM $MgCl_2$ (bottom) using the c-LEcta performance test* (see page 2). Measured DNA removal activities [$Unit_{Test}$/µL] were normalized by the applied nuclease units as specified in the manufacturer's CoAs [$Unit_{CoA}$/µL] and are depicted as [$Unit_{Test}/Unit_{CoA}$].

5.2 Magnesium

As with many 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 assess the impact of magnesium on the performance of DENARASE High Salt, enzyme activity was measured under standard test conditions in the presence of 0-100 mM $MgCl_2$. Requiring the presence of minimal magnesium levels for basal activity, DENARASE High Salt shows high activity at the standard condition of 5 mM $MgCl_2$ and pH 7.4 (**Fig. 3**; indicated by the dotted circle). Increasing the magnesium levels up to 25 mM and raising the pH value to 8.0 enhances the endonuclease activity across all tested salt concentrations (**Fig. 3** & **Fig. 4**). However, excess of $MgCl_2$ will reduce DENARASE High Salt activity (**Fig. 3**).

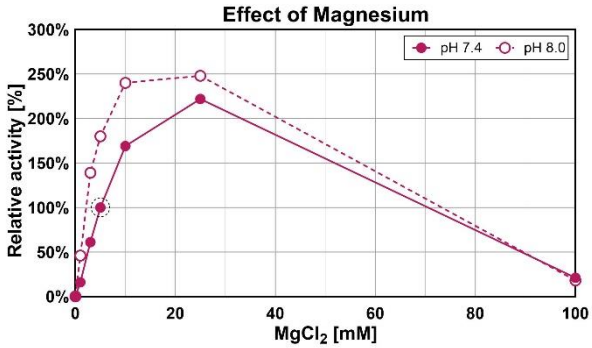

Fig. 3 Effect of increasing $MgCl_2$ concentrations on enzyme activity of DENARASE High Salt at 250 mM NaCl. DNA removal activities at pH 7.4 and pH 8 were measured in U/µL and normalized to activity at 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

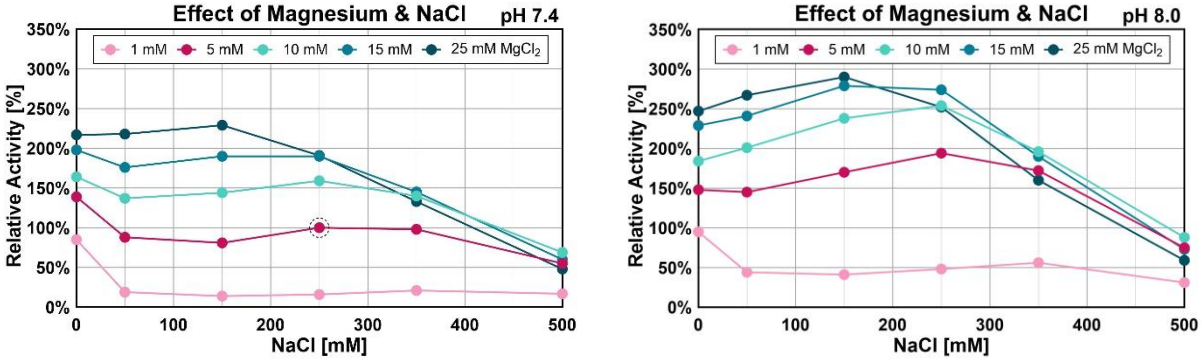

Fig. 4 Effect of low to intermediate $MgCl_2$ levels in combination with 0-500 mM NaCl on enzyme activity of DENARASE High Salt. Enzyme activities at pH 7.4 (left) and pH 8 (right) were measured in U/µL and normalized to activity at 250 mM NaCl, 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

DENARASE High Salt activity has been measured in frequently applied buffer systems such as Tris-HCl and phosphate buffers (**Fig. 5**). The data shows that DENARASE High Salt, similar to the wild-type enzyme, is inhibited by increasing phosphate concentrations. 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.

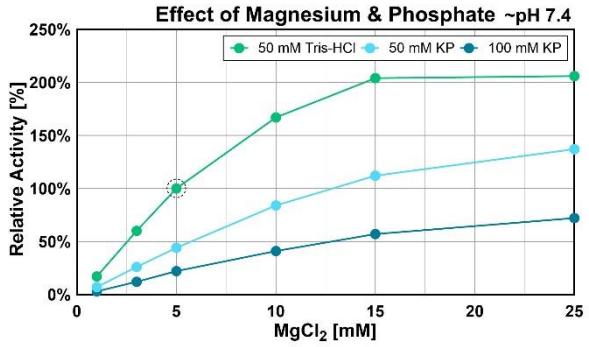

Fig. 5 Effect of $MgCl_2$ on DENARASE High Salt activity in Tris-HCl and potassium phosphate (KP) buffers. DNA removal activities at 250 mM NaCl were measured in U/µL and normalized to activity in 50 mM Tris-HCl at 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

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 $MgCl_2$) 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 tested pH levels compared to standard magnesium levels.

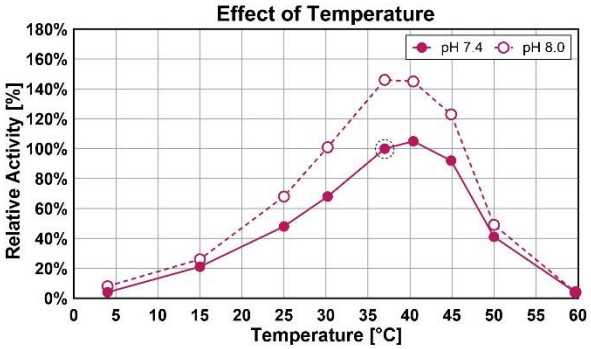

Fig. 6 Effect of temperature on DENARASE High Salt activity. Enzyme activities at different temperatures at pH 7.4 and 8.0 were normalized to activity at 37 °C, 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

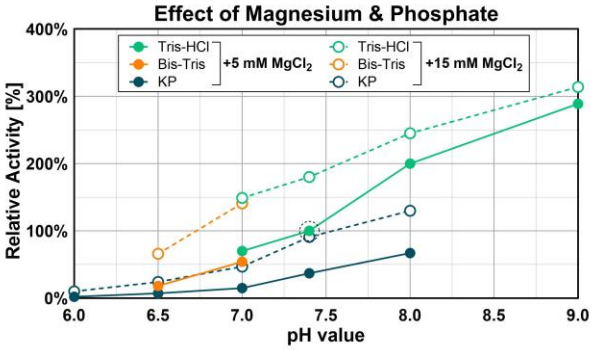

Fig. 7 Effect of pH on DENARASE High Salt activity. Enzyme activities in Tris-HCl, Bis-Tris and potassium phosphate (KP) buffer at different pH levels in the presence of 5 mM and 15 mM $MgCl_2$ were normalized to DENARASE High Salt activity at 5 mM $MgCl_2$ and pH 7.4 (indicated by dotted circle).

5.4 Detergents

Processing of different therapeutic biologics, including certain viral vectors, may require a cell lysis step to increase product recovery. For the lysis step, various commercially available non-ionic surfactants can be used to disrupt cellular membranes during the harvest step. To ensure that typical detergent concentrations do not interfere with DNA removal, the effect of frequently used detergents for cell lysis on DENARASE High Salt performance was evaluated using c-LEcta's test protocol (**Fig. 8**). Both Tween 20, Tween 80, and Triton® X-100 do not negatively impact the enzyme's activity when used up to 1%.

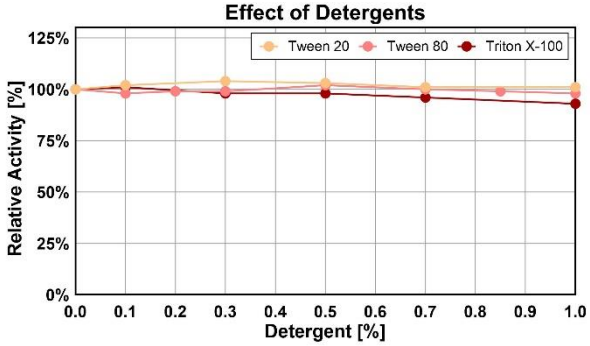

Fig. 8 Effect of different detergents on DENARASE High Salt activity at 250 mM NaCl and pH 8.0. DNA removal activities in the presence of 0-1% (v/v) of Tween 20, Tween 80 and Triton® X-100 were measured in U/µL and normalized to enzyme activity in the absence of the respective detergent.

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6 Stability & Storage Conditions

Based on stability data, DENARASE High Salt has a shelf life of at least 12 months when stored at the recommended storage temperature of -20 °C ± 5 °C. For the GMP-grade, this period is calculated from the date of manufacture, while for the R&D-grade, it is calculated from the date of product release. Long-term stability of DENARASE High Salt is under investigation.

Note: It is not recommended to store the product at -70 °C or below, since freezing the product will cause loss of activity.

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).

Parameter	Method	Specification
Appearance	Visual	Clear, transparent solution
Activity	Photometric[1]	> 250 U/µL
Purity	Protein purity determined by SDS-PAGE and silver staining	≥ 98 %
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/USP <85>, Method C	< 0.25 EU/kU
Total microbial count	TAMC/TYMC acc. to Ph. Eur. 2.6.12/USP <61>	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.

10 Sales and Contact

GMP Products for biopharmaceutical manufacturing

Product	Art. No	Size	Activity	
DENARASE High Salt 1 MU, GMP	22002-1M	1 MU	> 250 U/µL	Produced under EU GMP
DENARASE High Salt 5 MU, GMP	22002-5M	5 MU	> 250 U/µL	Produced under EU GMP

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

* Packaging units of the same size are indicated differently in the product name of DENARASE High Salt for biopharmaceutical manufacturing and DENARASE High Salt for research and development to avoid mix-ups (1000 kU DENARASE High Salt correspond to 1 MU DENARASE High Salt).

Contact

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