PRODUCT INFORMATION

DENARASE®

Recombinant *Serratia marcescens* endonuclease, liquid

DENARASE is the recombinant *Serratia marcescens* endonuclease produced by microbial fermentation with *Bacillus* sp. The enzyme cleaves all forms of DNA and RNA into smaller nucleotides of around 3-5 base pairs. This makes the enzyme attractive for the reduction of process related nucleic acids, such as host cell DNA and residual plasmids in biomanufacturing processes.

Typical Applications

An efficient and cost-effective removal of nucleic acids is crucial in biomanufacturing processes. DENARASE has been proven beneficial in a wide range of applications, such as

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

Compliance/Certificates

DENARASE is developed for use in commercial manufacturing processes of biologicals. Therefore, the enzyme is produced under GMP conditions acc. to EU GMP regulations without the use of antibiotics and materials with TSE/BSE risk and raw materials from animal origin.
The manufacturing of DENARASE and its distribution by c-LEcta is further compliant with the EXCiPACT and ANSI NSF 363 Standard and thus also meet the requirements for Good Manufacturing and Distribution Practices (GMP/GDP) for pharmaceutical excipients.
For GMP-grade DENARASE, dedicated regulatory support can be provided for US-market approvals of pharmaceutical products via a registered US FDA Drug Master File.

In addition, a DENARASE grade for research and development (R&D) use is available. R&D-grade DENARASE is produced in conformity with the ISO 9001 standard with less strict requirements regarding documentation, storage and distribution. This grade is suitable for R&D stages, when fast and easy access to raw materials is key. From a technical performance perspective, both quality grades are equal and the parameters on the specification are the same.

Removal of DENARASE

DENARASE endonuclease can be removed from the process intermediates with common purification technologies, such as chromatography and tangential flow filtration.

ELISA Kit

For the quantitative analysis of endonucleases from *Serratia marcescens* a DENARASE ELISA detection Kit is available.

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Operating conditions

DENARASE is a robust enzyme that is active under varying conditions. Similar to other enzymes, DENARASE activity is depending on various factors like temperature, pH and concentrations of cofactor and inhibitors.

Temperature/pH:
In order to determine the optimal reaction temperature and optimal pH value, DENARASE activity was measured under standard conditions at different temperatures and with different buffers at different pH values. The optimal reaction conditions for DENARASE are 37 °C at pH 8.0 – 9.0 (see Fig. 1, 2).

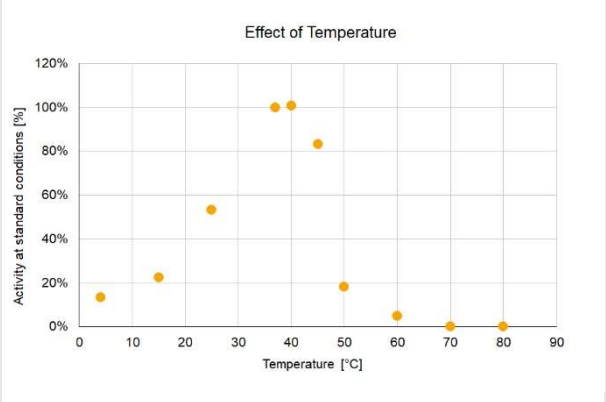

Fig. 1: Effect of Temperature. In order to determine optimal reaction temperatures, DENARASE activity was measured under standard conditions at different temperatures.
The optimal reaction temperature is 37 °C.
Temperatures above 40°C are not recommended.

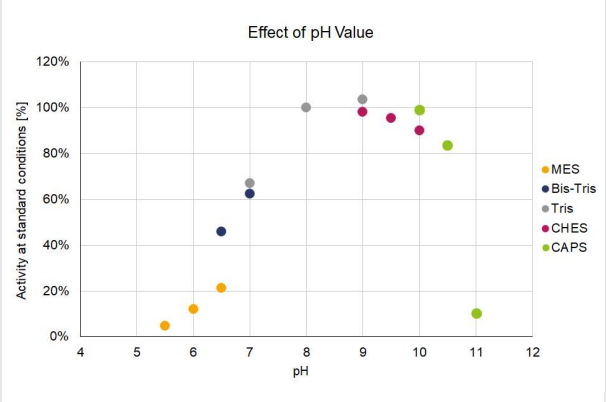

Fig. 2: Effect of pH value. In order to determine the pH optimum for DENARASE, the activity was measured with different buffers and at different pH values. DENARASE is highly active in nearly all tested buffer systems and shows a pH-optimum between pH 8.0 and 9.0.

Mg^{2+} Concentration:
The influence of high and low concentrations of $MgCl_2$ on DENARASE activity was measured under standard conditions. Mg^{2+} serves as a cofactor and a minimum amount is needed for enzyme activity. DENARASE requires 1- 2 mM Mg^{2+} cations for optimal activity (see Fig. 3). However, large excess of $MgCl_2$ reduces activity (see Fig. 4).

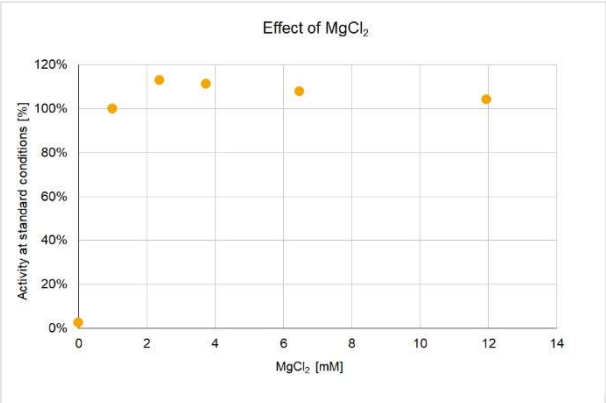

Fig. 3: The effect of low $MgCl_2$ concentrations on DENARASE activity.

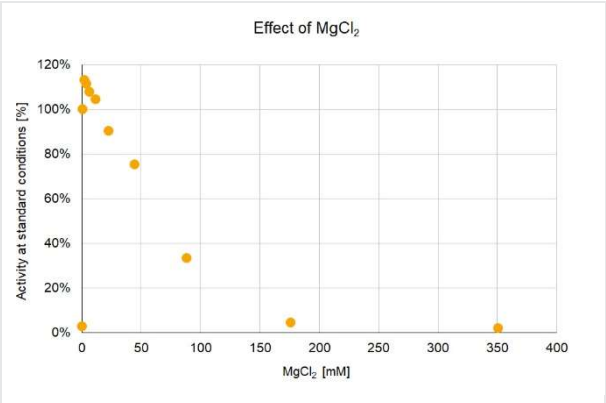

Fig. 4: The effect of high $MgCl_2$ concentrations on DENARASE activity.

Phosphate buffer:
DENARASE activity has been measured in frequently applied buffer systems such as Tris-HCl and phosphate buffers. The data shows that DENARASE activity is, in contrast to Tris-HCl, inhibited by increasing phosphate concentrations (see Fig. 5). However, this inhibiting effect can be circumvented by increasing the $MgCl_2$ concentration (see Fig. 6). If other buffers are used that may interact with Mg^{2+}, higher Mg^{2+} concentrations should be tested.

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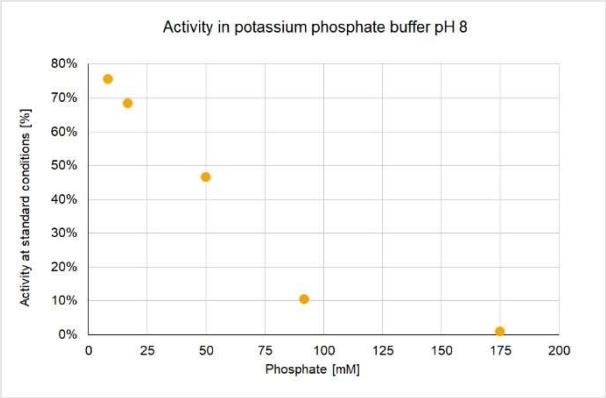

Fig. 5: Activity of DENARASE in potassium phosphate buffer pH 8.

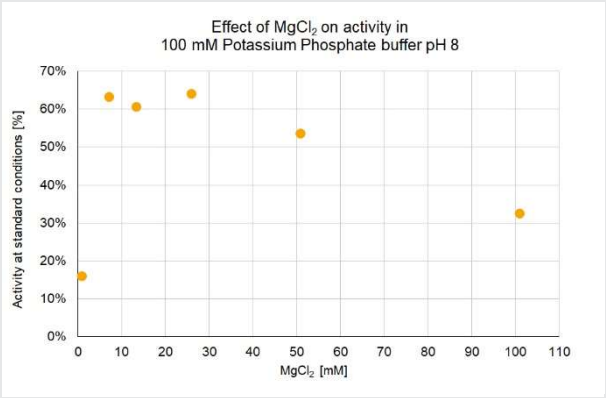

Fig. 6: Effect of $MgCl_2$ on DENARASE activity in 100 mM potassium phosphate buffer pH 8.

Monovalent Cation Concentration:
The presence of monovalent cations may inhibit DENARASE activity (see Fig. 7). Consequently, the concentration of monovalent cations such as Na^+ and K^+ should be kept below 200 mM for optimal DENARASE activity.

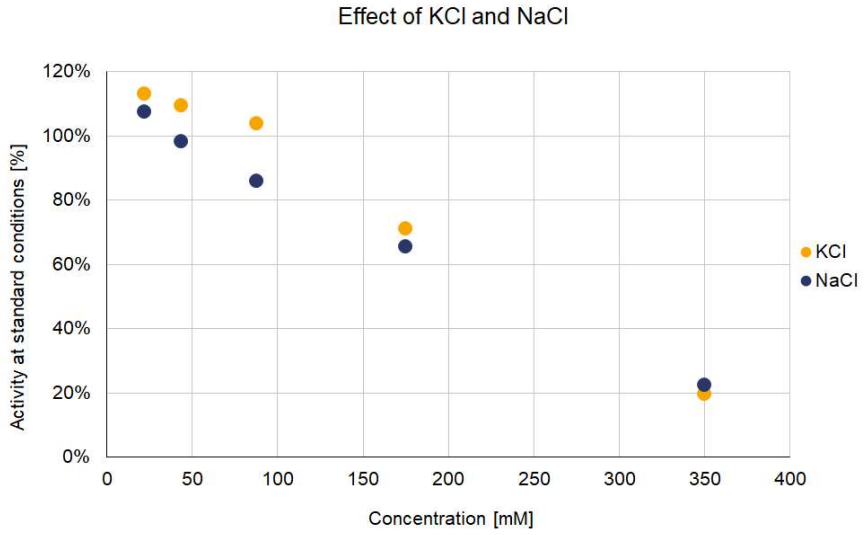

Fig. 7: Effect of KCl and NaCl on DENARASE activity. For different NaCl and KCl concentrations the activity of DENARASE was measured under standard conditions.

Antifoam:
DENARASE activity was measured in the presence of antifoam emulsion C to simulate applications in antifoam containing solutions, e.g. fermentation broth. Even at high concentrations (4 %) no inhibitory influence on DENARASE activity could be observed.

Stability and Storage Conditions

DENARASE is stable within specification range at a storage temperature of -20 °C ± 5°C for a period of at least 24 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.

Packaging Information

DENARASE is filled in non-pyrogenic, USP Class VI compliant vials. The product vials are shipped under qualified cooled conditions. Shipping temperature may differ from storage temperature without affecting product quality.

Enzyme Characteristics

The enzyme catalyses the hydrolysis of phosphodiesters of all forms of DNA and RNA like single-stranded, double-stranded, linear, circular or supercoiled forms into smaller nucleotides of mainly 3-5 base pairs.

Molecular weight (calculated)	27 kDa (per monomer)
pH optimum	pH 8.0 - 9.0
Temperature optimum	37 °C
Isoelectric point (pI, calculated)	pH 6.2
Cofactor	Mg^{2+}

Product Specification

DENARASE is the recombinant *Serratia marcescens* endonuclease 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 8.0 ± 0.2, 20 mM NaCl, 2 mM $MgCl_2$, 50 % glycerol (v/v).

In order to ensure a constant and high-quality level for DENARASE, each batch must fulfil the in-house acceptance criteria for the parameters listed below.

Criteria	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[2]	Activity per protein content determined photometrically at 280 nm with a molar extinction coefficient of 44,600 L x mol^{-1} x cm^{-1}	> 6 x 10^5 U/mg
Protease activity	Protease detection assay	No protease activity detectable
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.
[2] Vendor specifications for the specific activities of various commercially available endonucleases are not comparable due to differences in the activity assays and extinction coefficients.

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Sales and Contact

GMP products for biopharmaceutical manufacturing

Product	Art. No.	Size	Activity	
DENARASE 1 MU, GMP	20804-1M	1 MU	> 250 U/µl	Produced under EU GMP
DENARASE 5 MU, GMP	20804-5M	5 MU	> 250 U/µl	Produced under EU GMP

Products for use in research and development

Product	Art. No.	Size	Activity	
DENARASE 25 kU	20804-25k	25 kU	> 250 U/µl	Produced in conformity with ISO 9001 standard
DENARASE 100 kU	20804-100k	100 kU	> 250 U/µl	Produced in conformity with ISO 9001 standard
DENARASE 500 kU	20804-500k	500 kU	> 250 U/µl	Produced in conformity with ISO 9001 standard
DENARASE 1000 kU	20804-1000k	1 MU*	> 250 U/µl	Produced in conformity with ISO 9001 standard
DENARASE 5000 kU	20804-5000k	5 MU*	> 250 U/µl	Produced in conformity with ISO 9001 standard

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

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