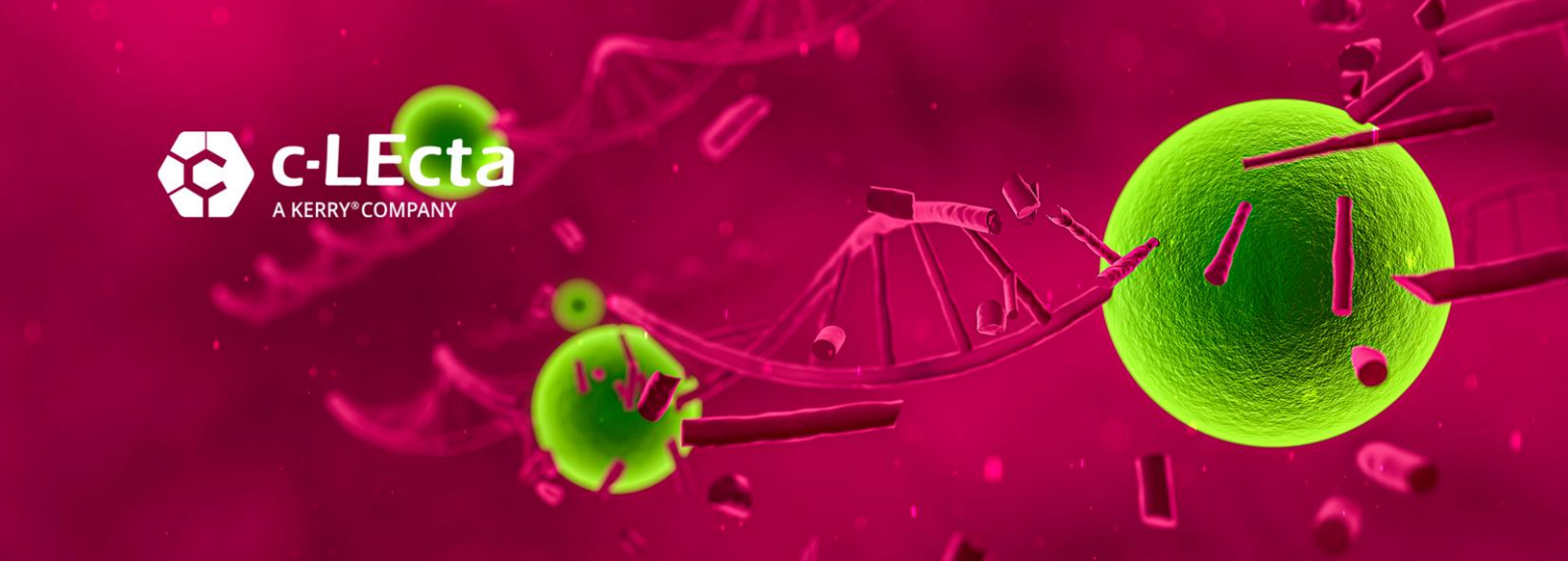

PRODUCT INFORMATION

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.

1 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.

2 Compliance

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.

3 Removal of DENARASE

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

4 ELISA Kit

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

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5 Operating conditions

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

5.1 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. DENARASE is highly active in nearly all tested buffer systems and shows a pH optimum between pH 8.0 and 9.0. The optimal reaction conditions for DENARASE are 37°C at pH 8.0-9.0 (**Fig**. 1, 2). Temperatures above 40 °C are not recommended, as they significantly impact DENARASE activity.

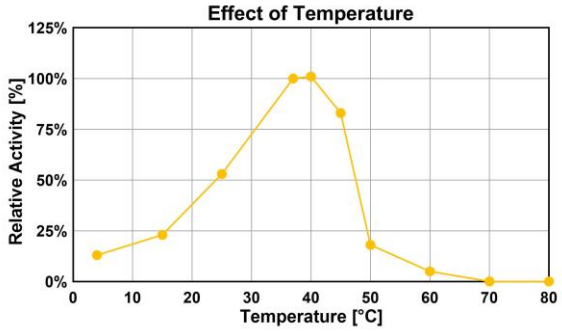

Fig. 1:. In order to determine optimal reaction temperatures, DENARASE activity was measured under standard conditions at different temperatures.
The optimal reaction temperature is 37 °C.

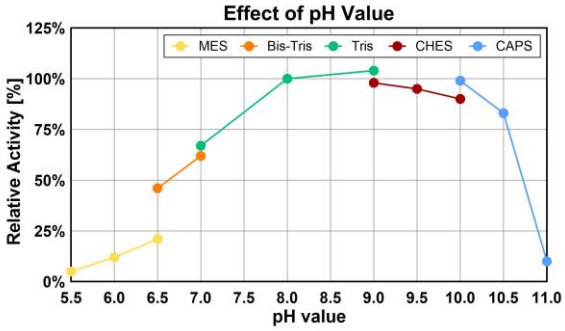

Fig. 2:. In order to determine the pH optimum for DENARASE, the activity was measured with different buffers and at different pH values.

5.2 Magnesium concentration

The influence of high and low concentrations of $MgCl_2$ on DENARASE activity was measured under standard conditions. Since Mg^{2+} serves as a cofactor, its presence is needed for DENARASE activity, while the optimum is 1-2 mM Mg^{2+} (**Fig**. 3). However, large excess of $MgCl_2$ reduces DENARASE activity (**Fig**. 4).

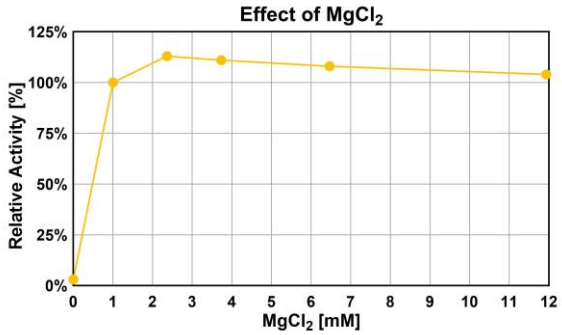

Fig. 3: The effect of low $MgCl_2$ concentrations on DENARASE activity.

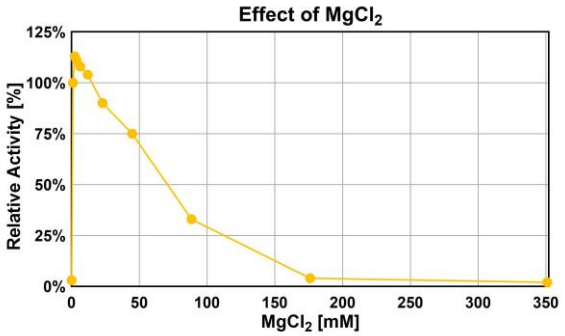

Fig. 4: The effect of high $MgCl_2$ concentrations on DENARASE activity.

5.3 Phosphate concentration

DENARASE activity has been measured in frequently applied buffer systems such as Tris-HCl and phosphate buffers. Increasing phosphate concentrations will inhibit DENARASE activity (**Fig**. 5). However, this inhibiting effect can be circumvented by increasing the $MgCl_2$ concentration (**Fig**. 6). For other buffers that may interact with Mg^{2+}, higher Mg^{2+} concentrations should be tested.

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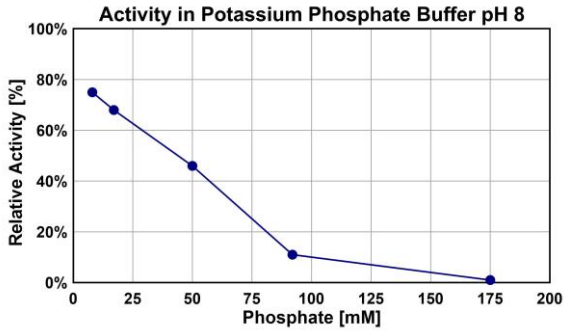

Fig. 5: Activity of DENARASE in potassium phosphate buffer pH 8.

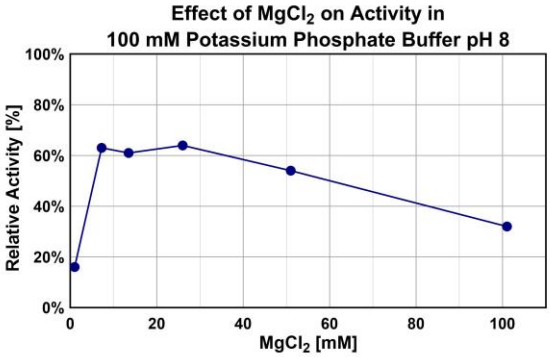

Fig. 6: Effect of $MgCl_2$ on DENARASE activity in 100 mM potassium phosphate buffer pH 8.

5.4 Monovalent Cation Concentration

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

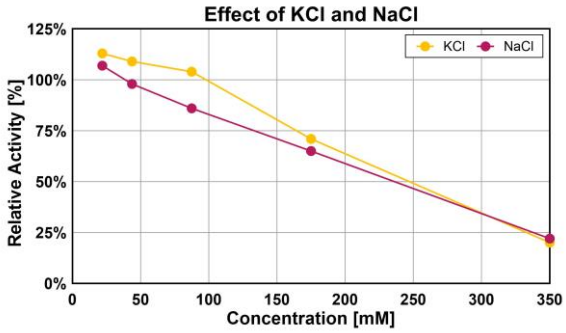

Fig. 7: For different NaCl and KCl concentrations the effect on DENARASE activity was measured under standard conditions.

5.5 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.

6 Stability and Storage Conditions

The stability of DENARASE Products has been determined and evaluated in accordance with the requirements of ICH guideline Q5C – Quality of Biotechnological Products: Stability Testing of Biotechnological/Biological Products and combined with long-standing product knowledge. The study comprises a batch-specific study plan with stability indicating profile, controlled and identical storage conditions, set deadlines and due dates for sampling and analysis, documented sampling and analysis and a final evaluation and release of the complete study. Based on this stability study with representative GMP batches, DENARASE Product stability* is given for a period of at least 36 months from the date of manufacture when stored at the recommended storage temperature of -20 °C ± 5 °C. Stability data are available in the current DENARASE Stability Report.

*The shelf life of DENARASE R&D-grade is at least 36 months from the date of product release when stored at the recommended storage temperature of -20 °C ± 5 °C.

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

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7 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. All DENARASE products will be delivered by c-LEcta in a sealed secondary packaging with tamper-evident seals.

8 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 [1]
Cofactor	Mg^{2+}

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

9 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[2]	> 250 U/µl
Purity	Protein purity determined by SDS-PAGE and silver staining	≥ 99 %
Specific Activity[3]	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

[2] 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.
[3] 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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10 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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