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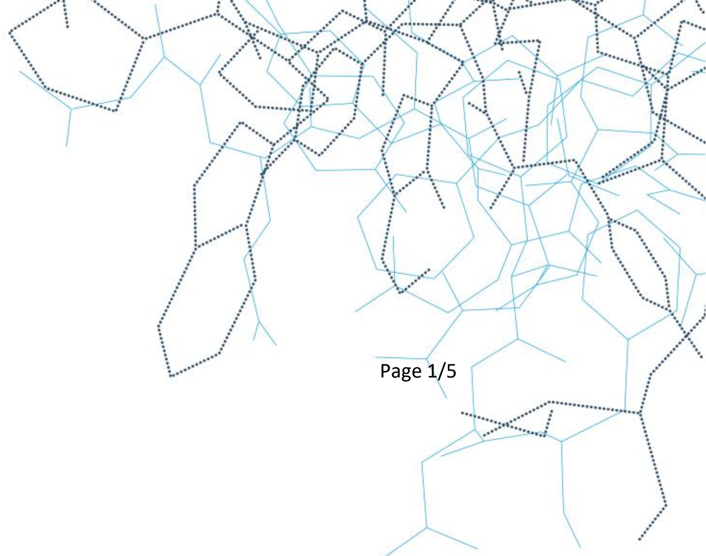


Product Information Sheet

DENARASE®

Recombinant *Serratia marcescens* endonuclease, liquid

valid as of February 28, 2020

Art. No.	Size	Activity	
20804-1M	1 MU	> 250 U/µl	Produced and filled under EU GMP
20804-5M	5 MU	> 250 U/µl	Produced and filled under EU GMP
		For manufacturing or uses requiring GMP reagents. Suitable for biopharmaceutical manufacturing.	
20804-100k	100 kU	> 250 U/µl	Produced under EU GMP, filled under ISO 9001
20804-500k	500 kU	> 250 U/µl	Produced under EU GMP, filled under ISO 9001
		The products are for research and development use/biotechnology applications only. Not for manufacturing or uses requiring GMP reagents.	

Recombinant *Serratia marcescens* endonuclease produced by microbial fermentation with a *Bacillus sp.* production host. The production strain employed in the manufacturing of the product is a Genetically Modified Organism (GMO) of safety level S1 and free of endotoxins.
The product is manufactured without the use of animal derived raw materials.

The enzyme is supplied as liquid and formulated in 20 mM Tris-HCl pH 8.2, 20 mM NaCl, 2 mM $MgCl_2$, 50 % glycerol (v/v).

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 oligonucleotides of mainly 2-5 base pairs.

Molecular weight (calculated)	27 kDa (monomer with two identical subunits)
pH optimum	pH 8.0 - 9.0
Temperature optimum	37 °C
Isoelectric point (pI, calculated)	pH 6.2
Cofactor	Mg^{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.

Compliance/ Certificates
The product is produced under GMP conditions acc. to EU GMP regulations. This product is further manufactured without the use of antibiotics and without the use of materials with TSE/BSE risk and raw materials from animal origin.

Typical Applications
In many industrial production processes of biopharmaceuticals and vaccines the efficient and cost-effective removal of nucleic acids is crucial and a strong regulatory demand. Especially in fermentation processes connected to cell disruption, large amounts of nucleic acids (RNA and DNA) are released.
c-LEcta's DENARASE provides a cost-efficient solution to these challenges in the purification of pharmaceutical products.

Operating conditions
DENARASE is a very 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).

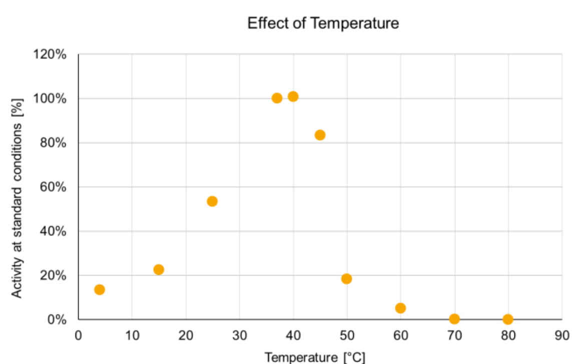


Fig. 1: Effect of Temperature. In order to determine optimal reaction temperatures, DENARASE activity was measured under standard conditions at different temperatures. The optimal reaction temperature is 37 °C. Temperatures above 40 °C are not recommended.

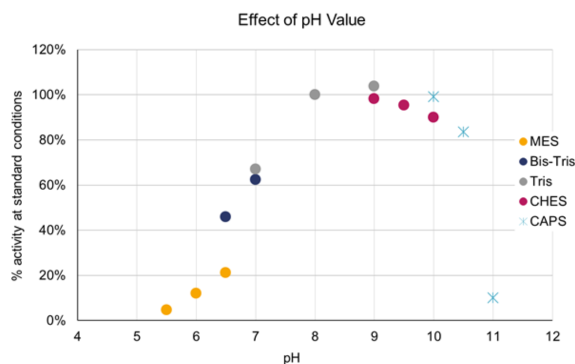
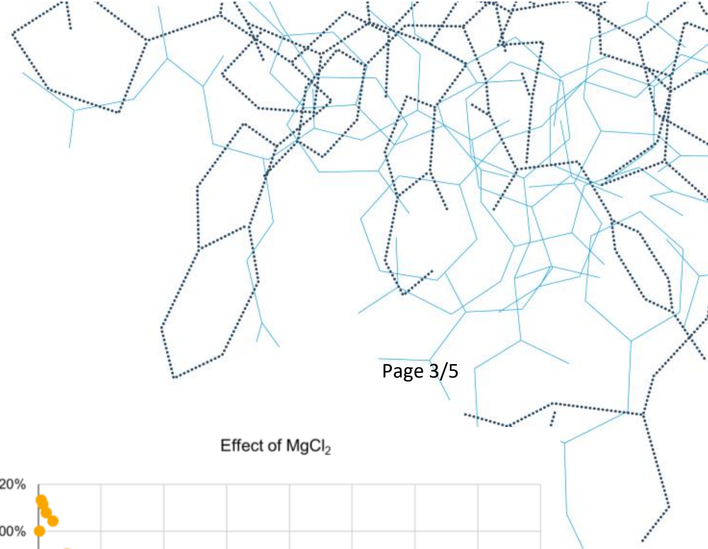


Fig. 2: Effect of pH value. In order to determine the pH optimum for DENARASE, the activity was measured with different buffers and at different pH values. DENARASE is highly active in nearly all tested buffer systems and shows a pH-optimum between pH 8.0 and 9.0.

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).



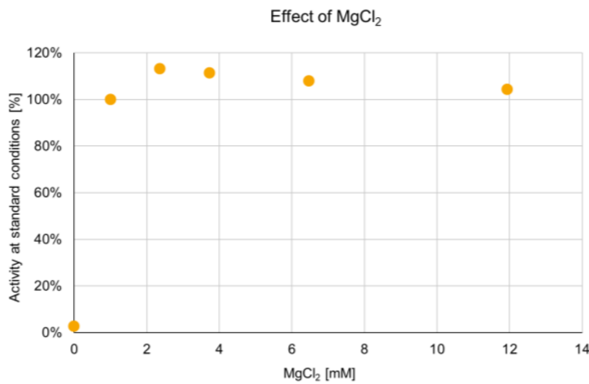


Fig. 3: The effect of low $MgCl_2$ concentrations on DENARASE activity.

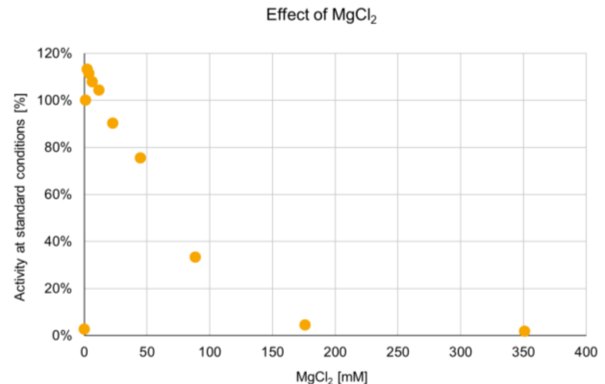


Fig. 4: The effect of high $MgCl_2$ concentrations on DENARASE activity.

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.

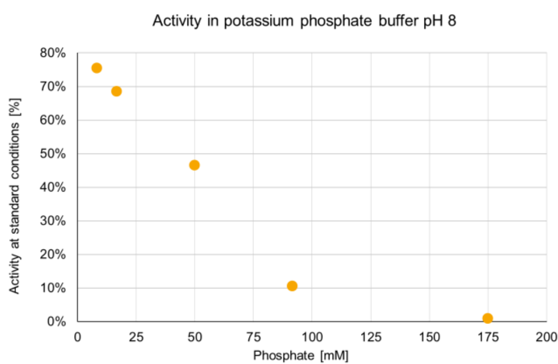


Fig. 5: Activity of DENARASE in potassium phosphate buffer pH 8.

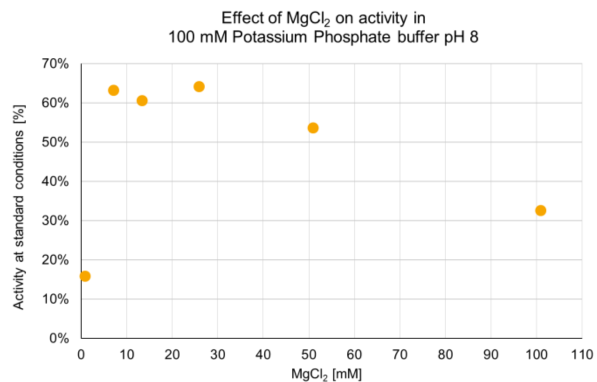


Fig. 6: Effect of $MgCl_2$ on DENARASE activity in 100 mM potassium phosphate buffer pH 8.

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.

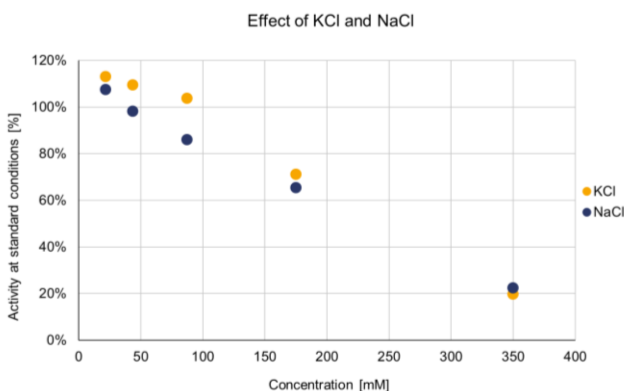


Fig. 7: Effect of KCl and NaCl on DENARASE activity.
For different NaCl and KCl concentrations the activity of DENARASE was measured under standard conditions.

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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 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 packed in polystyrene boxes and shipped under qualified cooled conditions. Shipping temperature may differ from storage temperature without affecting product quality.

Product Specifications

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	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.

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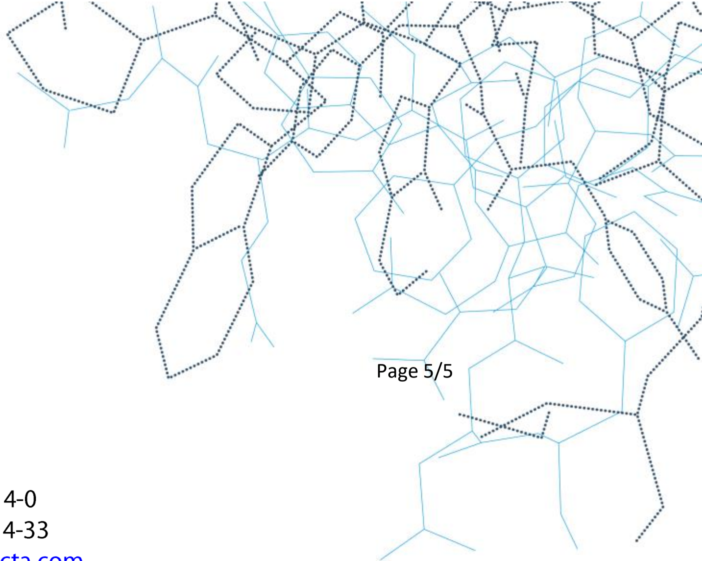

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