

Advancing Endonuclease Standardization in Biopharmaceutical Manufacturing

Implementation of USP General Chapter <89.3> Endonuclease (*Serratia marcescens*) for Denarase®

The Problem

- *Serratia marcescens* endonucleases such as Denarase® and Benzonase®* are industry standards for nucleic acid removal in biopharmaceutical manufacturing.
- Despite their identical amino acid sequence, activity values reported on different suppliers' CoAs cannot be directly compared due to vendor-specific activity assays.



The Solution

USP General Chapter <89.3> Endonuclease

- Provides standardized guidance for determining
 - volumetric activity
 - protein content
 - specific activity
- Improves comparability across commercially available endonucleases
- Endonuclease reference standard available (USP, Catalog No. 1235470)



The Adoption by Kerry

Implementation of Denarase®

- Kerry has implemented the USP <89.3> assay as an additional activity test for Denarase® to support standardized comparison of commercially available *Serratia marcescens* endonucleases.
- Activity [U/μL] and specific activity [U/mg] determined according to USP <89.3> will be added to Denarase® CoAs.
- Parameters according to USP <89.3> will be reported as additional information and not be used for batch release.
- The established release assay, specifications and acceptance criteria will remain unchanged.



See the example CoA on pages 2 and 3.

USP <89.3> testing is performed in-house using validated methods within an EXCiPACT® GMP-certified quality management system.

*Benzonase nuclease is a registered trademark of Merck KGaA

Denarase 1 MU, GMP

Recombinant *Serratia marcescens* endonuclease, liquid

Art. No.	Lot. No.	Date of manufacture	Best before
20804-1M	GD-a-XXXX	Month DD, YYYY	Month DD, YYYY

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 MgCl₂, 50 % glycerol (v/v).

The product is produced under EU GMP.

Criteria	Method	Specification	Result
Appearance	visual	Clear, transparent solution	compliant
Activity	photometric ¹	> 250 U/μl	XXX U/μl
Purity	Protein purity determined by SDS-PAGE and silver staining	≥ 99 %	compliant
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	X x 10 ⁵ U/mg
Protease activity	Protease detection assay	No protease activity detectable	compliant
Endotoxin level	LAL-Test acc. to Ph. Eur. 2.6.14, Method C	< 0.25 EU/kU	compliant
Total microbial count	TAMC/TYMC acc. to Ph. Eur. 2.6.12	Aerobic bacteria: < 5 cfu/200 μl Yeast/moulds: < 5 cfu/200 μl	compliant

¹ Unit-Definition: One unit (U) will digest salmon sperm DNA to acid-soluble oligonucleotides equivalent to a ΔA_{260nm} 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.

Additional Information: USP <89.3> Activity Parameters (non-release testing)

Criteria	Method	Result
Activity (USP)	photometric ^{3,4} acc. to USP <89.3>	XXX U/μl
Specific Activity (USP)	Activity per protein content determined photometrically using the Bradford assay in accordance with USP <507>, as referenced in USP <89.3> ⁴	X x 10 ⁶ U/mg

³ Unit-Definition: A unit (U) of endonuclease (*S. marcescens*) is defined as the amount of enzyme that produces an absorbance change of 1.0 in 30 min at 260 nm under the conditions provided in USP <89.3>.

⁴ Tested at Kerry Biotechnology Centre / c-Lecta GmbH, Alte Messe 3, 04103 Leipzig, Germany, under an EXCiPACT GMP-certified Quality Management System.

Storage: Store at -20 °C ± 5 °C.

BSE / TSE / Animal derived material: The manufacturing process is free of materials with TSE/BSE risk and of raw materials from animal origin.

GMO-Statement: The product is free of the production strain.

Antibiotics: No antibiotics are used in the manufacturing process.

Date

Reviewed by Quality Assurance

Date

Approved by Head of QC

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Version X.X, valid as of XX. XX, 20XX

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