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DATASHEET

Benz-Nebulase™ GMP, tag free

Cat. No.: Z03708

Table of Contents

GenScript USA, Inc.
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I. Product Introduction

Benz-Neburase™ GMP, tag-free is a genetically engineered endonuclease from *Serratia marcescens* that cleaves all forms of DNA and RNA non-specifically under a broad range of conditions. Benz-Neburase™ Nuclease is mostly utilized to remove residual DNA and RNA during production process of biological molecules of interest in biopharmaceutical manufacturing including but not limited to vaccine production, viral vector production, as well as in gene and cell therapy-related manufacturing.

Benz-Neburase™ GMP, tag free (Cat. No. Z03708) is manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards and with more stringent process controls and relatively complete document records. The high-purity nucleases are suitable and fulfill the requirements for applications from basic research to clinical settings.

Product Name	Benz-Neburase™ GMP, tag free
Species	*Serratia marcescens*
Expression System	*E.coli*
Tag	Tag free
Theoretical Molecular Weight	27.0 kDa
Activity	≥ 250 U/μl (or 1.1×10^6 U/mg) One unit is defined as the amount of enzyme that digests sonicated salmon sperm DNA to acid-soluble oligonucleotides equivalent to a ΔA260 of 1.0 in 30 min at pH 8.0 at 37 °C (within reaction volume 0.5 ml).
Purity	SEC-HPLC: ≥ 99% Reducing SDS-PAGE: ≥ 95%
Endotoxin Level	< 0.01 EU/μg
Applications	Digests all forms of DNA and RNA (double stranded, single stranded, linearized, and circular forms) under a board range of conditions.
Storage Buffer	20 mM Tris-HCl, 2 mM $MgCl_2$, 20 mM NaCl, 50% Glycerol, pH 8.0.
Storage & Stability	Store at -20 °C for up to 24 months or at 4 °C for 2 weeks from the date of manufacture. Avoid repeated freeze-thaw cycles. ***Do not store below -20 °C!***

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II. Reagents Provided

Contents	Cat. No.	Amount	Storage
Benz-Neburase™ GMP, tag free	Z03708-10	10 kU	-20 °C
	Z03708-100	100 kU	
	Z03708-500	500 kU	

III. Protocols

i. Recommended Reaction Conditions

Condition	Optimal*	Effective**
Mg^{2+}	1-2 mM	1-10 mM
pH	8.0-9.0	5.0-11.0
Temperature	37-45 °C	25-55 °C
Salt ions (Na^+, K^+, etc.)	0-20 mM	0-300 mM
PO_4^{3-}	0-10 mM	0-40 mM
Urea	4 M	0-6 M
SDS	SDS inactivates Benz-Neburase™ Nuclease in 10 min at any concentration.	

*Optimal conditions are defined when the nuclease retains over 90% of its activity.

**Effective conditions are defined when the nuclease retains over 15% of its activity

ii. Recommended Applications and Amount

Assay Type	Protein Expression	Viral Vector or Vaccine Production	Prevent from Cell Clumping
Sample amount	1-20 ml lysis buffer per gram of wet weight (~10^9 cells)	1 L supernatant/lysis buffer	/
Recommended amount of Benz-Neburase™ GMP, tag-free	> 250 U	~20000 U	50 U/ml
Incubation time	Generally incubate at 37 °C for 30-60 min.		

iii. Assay Procedures for Removal of Plasmid and Host Residual DNA during Lentivirus (LV) Production

Steps	Operations
Step 1	Dilute Benz-Neburase™ Nuclease with cell culture medium to 10 kU/ml and place it in a chromatography refrigerator at 4 °C for later use.
Step 2	Mix the harvested cell suspension (5 ml) and add 10 µl of Benz-Neburase™ Nuclease mix thoroughly and place in a 37 °C water bath for 60 min.
Step 3	After the incubation, centrifuge at 1300 g for 10 min to remove the cells and cell debris.
Step 4	After the centrifugation, measure the HCD, plasma residues in the samples. Fluorescence activated Cell Sorting (FACS) can be used to determine the functional titer of lentivirus particles. Lentivirus Titer p24 ELISA Kit (Cat. No. L00938, GenScript) can be used to determine the physical titer of lentivirus particles.

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iv. Assay Procedures for Removal of Plasmid and Host Residual DNA during Adeno Associated Virus (AAV) Production

Steps	Operations
Step 1	After harvesting cell suspension, break up the cells, then add 100 U Benz-Neburase™ Nuclease to 2 ml of cell suspension, mix thoroughly and place in a 37°C water bath for 60 min.
Step 2	After the incubation, centrifuge to remove the cells and cell debris at 1600 g for 10 min.
Step 3	After the centrifugation, measure the HCD and plasmid DNA residues in the samples. Viral genome (Vg) recovery can be used to quantify the adeno associated viral production.

v. Assay Procedures for Reduction of Viscosity of Bacterial Lysate

Steps	Operations
Step 1	Centrifuge the bacterial culture, remove the supernatant, and then add the lysate.
Step 2	Treat the sample with Benz-Neburase™ Nuclease at a final concentration of 2.5 U/ml, incubate at 37 °C for 30 min.
Step 3	Centrifuge to observe the viscosity of the precipitate and the supernatant.

vi. Assay Procedures for Prevention of Cell Clumping

Steps	Operations
Step 1	Spread the adhered cells in a 24-well plate and treat them 50 U/ml Benz-Neburase™ Nuclease at 37 °C for 30 min separately.
Step 2	Observe the cells using a microscope

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Appendix 1: Quality Control Specifications

Benz-Neburase™ GMP, tag free (Cat. No. Z03708) is manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards and with more stringent process controls and relatively complete document records. Benz-Neburase™ GMP, tag free (Cat. No. Z03708) meets the following quality control specifications.

Assay	Specifications
Appearance	Clear, colorless liquid
Specific Activity	≥ 1.1×10^6 U/mg
Enzyme Activity	≥ 250 U/μl
Purity	≥ 99% as analyzed by SEC-HPLC
	≥ 95% as analyzed by reducing SDS-PAGE
Endotoxin Level	< 0.01 EU/μg
Residual HCP	≤ 20 ng/mg
Protease Activity	Non-detectable
Bioburden	< 1 CFU/ml
Mycoplasma	Negative

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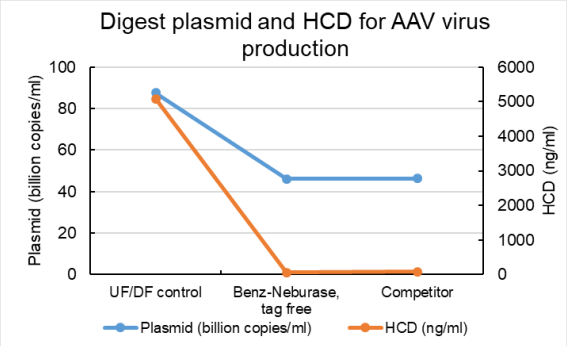
Appendix 2: Case Studies

i. Removal of plasmid and host residual DNA during adeno associated virus (AAV) production



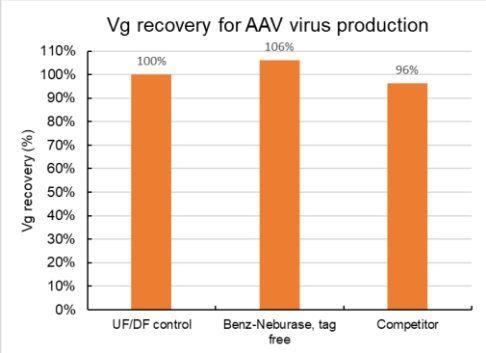


Figure 1: Benz-Neburase™ Nuclease removes nucleic acid residues during AAV production. 100 U Benz-Neburase™ GMP, tag free (Cat. No. Z03708) was used to treat 2 ml of cell suspension at 37 °C for 60 min. Then the mix was centrifuged at 1600 g for 10 min to remove cells and cell debris, and measurements were taken for the HCD, plasmid DNA residues, and viral genome (Vg) recovery. The test results show that Benz-Neburase™ GMP, tag free can remove DNA and plasmid residue in AAV virus production process more effectively than the competing product. The usage of Benz-Neburase™ GMP, tag free has minimal impact on viral production.

ii. Reduction of viscosity of bacterial lysate

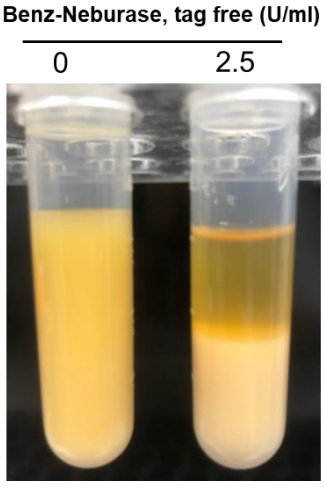


Figure 2: Benz-Neburase™ Nuclease reduces viscosity of bacterial lysate by digesting nucleic acid. 2.5 U Benz-Neburase™ GMP, tag free (Cat. No. Z03708) was used to treat 1 ml of bacterial lysate. Incubate at 37 °C for 30 min and then centrifuge the samples and observe the viscosity of the precipitate and the supernatant. The test results show that Benz-Neburase™ GMP, tag free can greatly reduce the viscosity of bacterial lysate.

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iii. Prevention of cell clumping

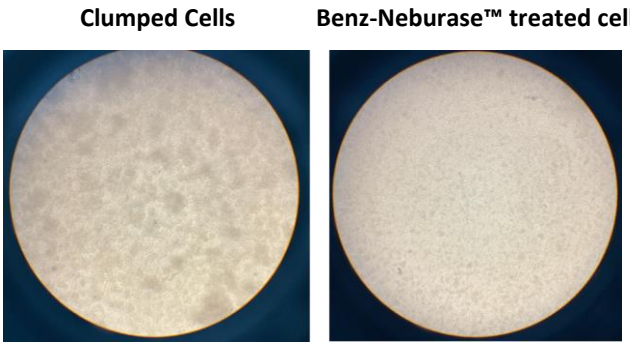
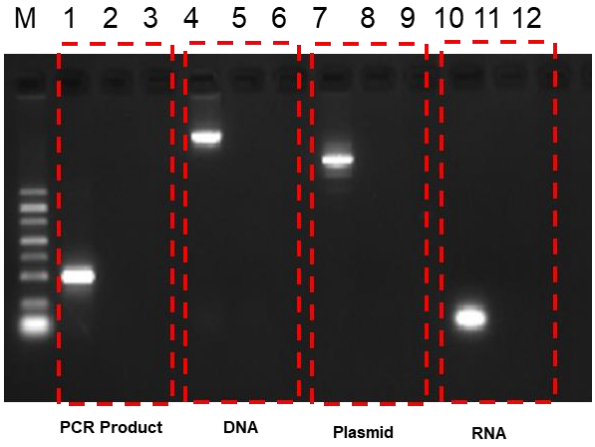


Figure 3: Benz-Neburase™ Nuclease prevents and reduces cell clumping. The adhered cells were spread in a 24-well plate and treated with control buffer (left) and 50 U/ml Benz-Neburase™ GMP, tag free (Cat. No. Z03708) (right) at 37 °C for 30 min. After the incubation, the cells were observed under a microscope. The test result show that Benz-Neburase™ GMP, tag free can efficiently reduce cell clumping.

iv. Digestion of various types of nucleic acids



Lane M: DNA marker
Lane 1: PCR Product
Lane 2: Benz-Neburase + PCR Product
Lane 3: Competitor endonuclease + PCR product
Lane 4: Genomic DNA
Lane 5: Benz-Neburase + Genomic DNA
Lane 6: Competitor endonuclease + Genomic DNA
Lane 7: Plasmid DNA
Lane 8: Benz-Neburase + Plasmid DNA
Lane 9: Competitor endonuclease + Plasmid DNA
Lane 10: RNA
Lane 11: Benz-Neburase + RNA
Lane 12: Competitor endonuclease + RNA

Figure 4: Benz-Neburase™ Nuclease digests various types of nucleic acids. In a 20 µl reaction volume, 1 U of Benz-Neburase™ GMP, tag free (Z03708) was used to digest different kinds of nucleic acids (PCR product, genomic DNA, plasmid DNA, and RNA) at 37 °C for 10 min. The test results show that Benz-Neburase™ GMP, tag free is effective in digesting various forms of DNA and RNA.

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FAQs

1. **How to enhance the nucleic acid removal efficiency of Benz-Neburase™ Nuclease if the reaction setup has suboptimal conditions?**
   The removal efficiency of Benz-Neburase™ Nuclease depends on the working concentration of the enzyme, incubation time, reaction temperature and other conditions. If the salt, pH, temperature or other reaction conditions are not optimal, increasing the working concentration of the Benz-Neburase™ Nuclease and/or extending the reaction incubation time would improve the digest efficiency.

2. **Is it necessary to add Mg^{2+} when adding Benz-Neburase™ Nuclease?**
   Benz-Neburase™ Nuclease has the highest activity in the presence of 1-2 mM Mg^{2+}. If the concentration of the magnesium ion in the reaction is low, it is recommended to supplement with more Mg^{2+} to achieve the optimal reaction concentration of 1-2 mM.

3. **At which step should Benz-Neburase™ Nuclease be added?**
   It depends on the application scenario and assay design. However, Benz-Neburase™ Nuclease is recommended to be added after the cultivation step and before the capture step.

4. **How to remove Benz-Neburase™ Nuclease?**
   The removal of Benz-Neburase™ Nuclease can be accomplished by several downstream units of operation, such as depth filtration for clarification, tangential flow filtration (TFF) for concentration, and diafiltration and chromatography (IEX, SEC, HIC).

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