Version: 03
Update: 08/24/2023

DATASHEET

Benz-Neburase™ GMP, His-tag

Cat. No.: Z03627-10; Z03627-100; Z03627-500
Size: 10 kU; 100 kU; 500 kU

Product Introduction

Benz-Neburase is a genetically engineered endonuclease from *Serratia marcescens* used in the purification processes of biological molecules and biopharmaceutical production such as vaccine, viral vector, gene and cell therapy manufacturing facilities. The enzyme is highly effective, nonspecific endonuclease capable of digesting all forms of DNA and RNA into 3-5 bp nucleotides.

Benz-Neburase is active in a broad range of conditions and is free of proteolytic activity, making the enzyme especially useful for applications with challenging DNA contaminants, such as lysed host cells in viral vector manufacturing processes. The nuclease requires divalent cation, preferably Mg^{2+} for activity, displays a broad pH tolerance, ranging from pH 6 to pH 10, optimal at 8-8.5, and has a wide temperature tolerance, ranging from 35 °C to 55 °C. Benz-Neburase is a physiologic homodimer and functions more progressively than the monomer. Two disulfide bonds in the nuclease are crucial to its activity and stability.

GenScript's offerings are cost-effective with reliable and proven performance for removal of contaminating nucleic acids. Furthermore, the Benz-Neburase's offerings are produced under stringent quality controls with materials and manufacturing process that are fully traceable, allowing for support of IND filing and progression of development stages for various applications.

Product Name	Benz-Neburase™ GMP, His-tag
Species	*Serratia Marcescen*
Expression System	*E.coli*
Tag	N-terminal 6 × His
Theoretical Molecular Weight	27.5 kDa
Specific Activity	≥ 1.1 × 10^6 U/mg One unit of Benz-Neburase™ GMP, His-tag is defined as the amount of enzyme that causes a ΔA260 of 1.0 (equivalent to the complete digestion of 37 μg DNA) in 30 min.
Enzyme Activity	≥ 250 U/μl
Purity	SEC-HPLC: ≥ 99% Reducing SDS-PAGE: ≥ 95%
Endotoxin Level	≤ 0.01 EU/kU
Application	This enzyme requires 1-2 mM Mg^{2+} to be used to remove all forms of DNA and RNA, including double stranded, single stranded, linearized, and circular forms.
Formulation	Supplied as a solution of 20 mM Tris-HCl, 2 mM $MgCl_2$, 20 mM NaCl, 50% Glycerol, pH 8.0.
Storage & Stability	This product remains stable for up to 2 weeks at 4 °C or up to 30 months at -20 °C. Avoid repeated freeze-thaw cycles. ***Do not store below -20 °C!***

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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/kU
Residual HCP	≤ 10 μg/mg
Protease Activity	Non-detectable
Bioburden	< 1 CFU/ml
Heavy Metal Residue	≤ 10 ppm
Mycoplasma	Negative

Protocols for Various Applications

1. Recommended reaction conditions

Condition	Optimal*	Effective**
Mg^{2+}	1-2 mM	1-10 mM
pH	8.0-9.2	5.0-11.0
Temperature	37 °C-45 °C	25 °C -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 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

2. Recommended amount of Benz-Neburase used for various applications

Assay type	Protein Manufacturing	Virus/Vaccine Purification	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	> 250 U	~20000 U	50 U/ml
Incubation time	Generally incubate at 37 °C for 30-60 min.		

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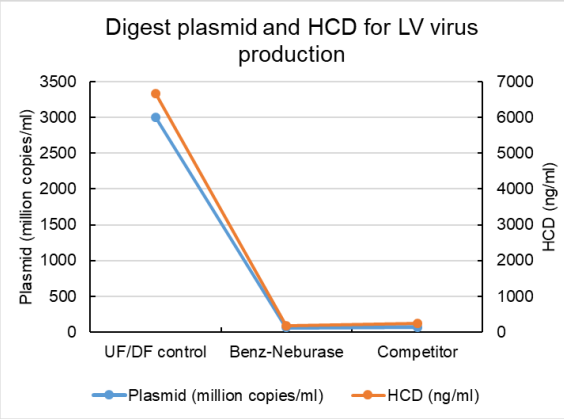
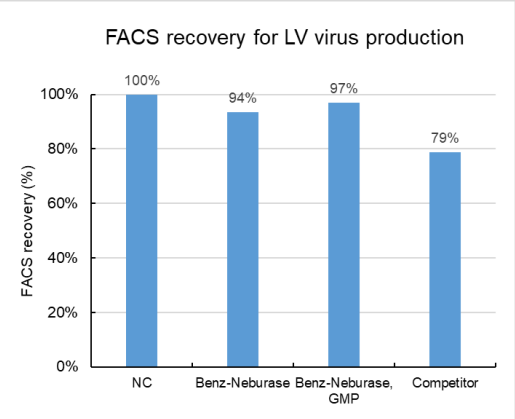
3. Data Images

3.1 Removal of plasmid and host residual DNA during Lentivirus (LV) production





Step 1: Dilute Benz-Neburase to 10 kU/ml and place it in a chromatography refrigerator at 4 °C for later use.

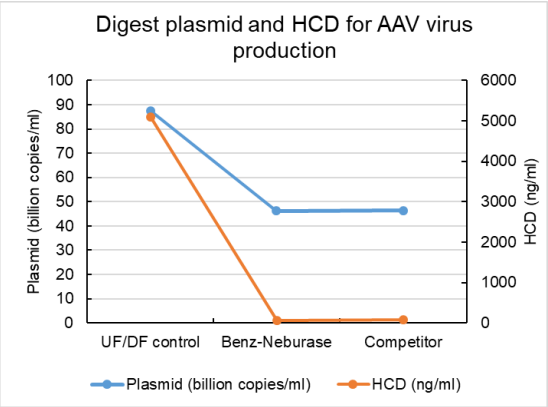
Step 2: Mix the harvested cell suspension (5 ml) and add 10 µl Benz-Neburase, mix thoroughly and place in a 37 °C water bath for 60 min.

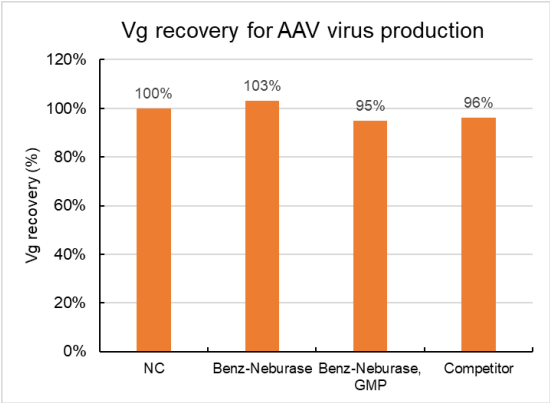
Step 3: After the incubation, centrifuge at 1300 g for 10 min to remove the cells and cell debris.

Step 4: After the centrifugation, measure the HCD, plasma residues in the samples, and Fluorescence activated Cell Sorting (FACS) can be used to determine the functional titer of lentivirus particles.

The test results show that Benz-Neburase can remove DNA and plasmid residue in virus production process more effectively than the competitor product. The use of Benz-Neburase has minimal impact on the viral production recovery. The recovery data further indicates that the GenScript Benz-Neburase outperforms the competitor product.

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





Step 1: After harvesting cell suspension, break up the cells, then add 100 U Benz-Neburase to 2 ml 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, and Viral genome (Vg) recovery was used to quantify the adeno associated viral production.

The test results show that Benz-Neburase can also remove DNA and plasmid residue in AAV virus

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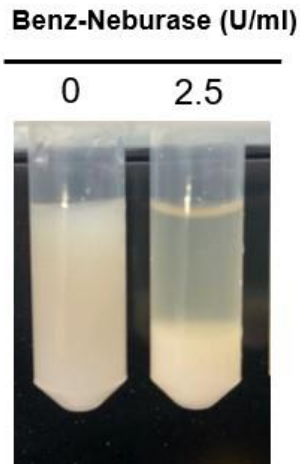
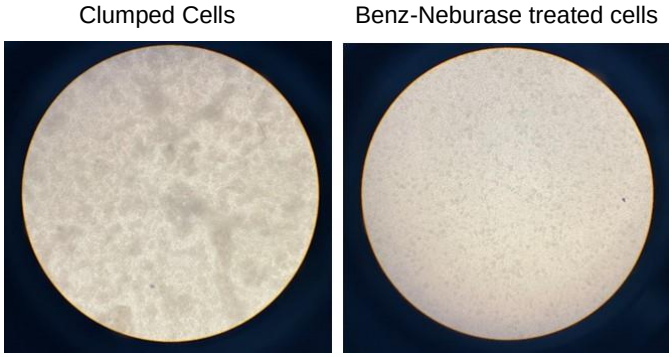
production process more effectively than the competitor product. The use of Benz-Neburase has minimal impact on the viral production. The data further indicates that the GenScript Benz-Neburase outperforms the competitor product

3.3 Reduction of the viscosity of bacterial lysate



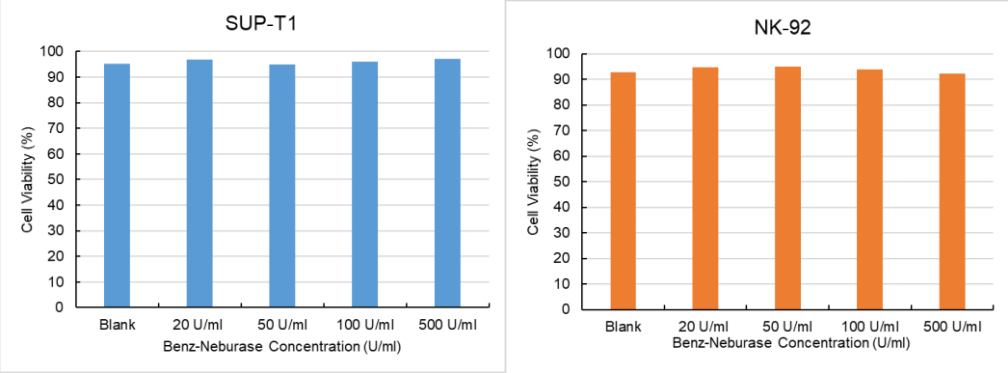
Step 1: Centrifuge the bacterial culture, remove the supernatant, and then add the lysate.
Step 2: Treat the sample with Benz-Neburase at a final concentration of 2.5 U/ml, incubate at 37 °C for 30 min.
Step 3: Centrifuge to observe the viscosity of the precipitate and supernatant.
The test results show that Benz-Neburase can greatly reduce the viscosity of bacterial lysate.

3.4 Prevention of cell clumping



Step 1: Spread the adhered cells in a 24-well plate and treat them with control buffer (left) and 50 U/ml Benz-Neburase (right) at 37 °C for 30 min.
Step 2: Observe the cells using a microscope

The test result show that Benz-Neburase can efficiently reduce cell clumping.



Step 1: Treat SUP-T1 cells (left) and NK-92 cells (right) with 2 µl of Benz-Neburase at different concentrations (20-500 U/ml).
Step 2: Incubate overnight in an incubator at 37 °C in 5% CO_2.
The test results indicate Benz-Neburase has minimal to no impact on the cell viability.

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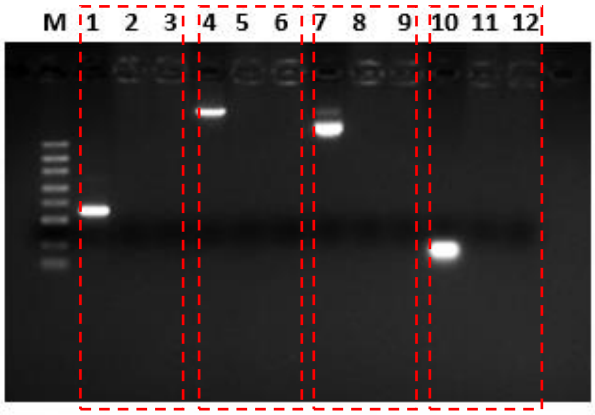
3.5 Digestion of various types of nucleic acids



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

In 20 µl reaction volume, use 1U of Benz-Nebulase to digest different kinds of nucleic acid at 37 °C for 10 min.

The test results show that Benz-Nebulase is effective in digesting various forms of DNA and RNA, with efficiency identical to competing products.

FAQs

1. **How to enhance the nucleic acid removal efficiency of the nuclease if the reaction setup has suboptimal conditions?**
   The removal efficiency of Benz-Nebulase 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-Nebulase and/or extending the reaction incubation time would improve the digest efficiency.
2. **Is it necessary to add more Mg^{2+} when adding nuclease?**
   The Benz-Nebulase 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 more Mg^{2+} to achieve the optimal reaction concentration of 1-2 mM.
3. **At which step should the Benz-Nebulase be added?**
   It depends on the application scenario and assay design. However, it is recommended for the Benz-Nebulase to be added after the cultivation and before the capture step.
4. **How to remove Benz-Nebulase?**
   Removal of Benz-Nebulase can be accomplished by several downstream units of operation, like depth filtration for clarification, tangential flow filtration (TFF) for concentration and diafiltration and chromatography (IEX, SEC, HIC).

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