

# Benz-Neburase™

A genetically engineered endonuclease from *Serratia marcescens* for nucleic acid removal



Powerful



Efficient



Pure



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## Ordering Information

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## 1.1 Benz-Neburase™ overview

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### Overview

GenScript's Benz-Neburase™ is a highly effective, nonspecific, genetically engineered endonuclease capable of digesting all forms of DNA and RNA including double stranded, single stranded, linearized, and circular forms. The resulting nucleic acid fragments are 3-5 base pairs in size. This endonuclease is commonly used in biopharmaceutical production of vaccines, viral vectors, and gene and cell therapy products.

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**The Benz-Neburase product line provides customers with the following two tiers of nuclease grades to choose from:**

- » Research use only (RUO)
- » Good Manufacturing Practice (GMP)

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The RUO products provide cost-effective solutions for basic research applications. With stringent quality controls, ISO9001, ISO13485 and GMP manufacturing guidelines, the GMP products are ideal solutions for pre-clinical and clinical applications.

## 1.2 Benz-Neburase™ product features

### Overview

#### Non-specific endonuclease activity:

- Benz-Neburase non-specifically digests all forms of DNA and RNA enabling removal of contaminating nuclease acids in laboratory applications and manufacturing facilities of biological products.

**High purity:** ≥ 99% by SEC-HPLC

**High activity:** Specific activity\* ≥  $1.1 \times 10^6$  U/mg

- Safe:**
- Complies to the United States Pharmacopeia (USP) standards, animal-free and ampicillin-free
  - Low Endotoxin Level: ≤ 0.01 EU/kU
  - Benz-Neburase™, tag-free is also available

**High manufacturing standard:** Compliant to GMP regulations, all raw materials and records are lot traceable

\*Activity: One unit of Benz-Neburase is defined as the amount of enzyme for a  $\Delta$ A260 of 1.0 (equivalent to the complete digestion of 37 $\mu$ g DNA) in 30 min.

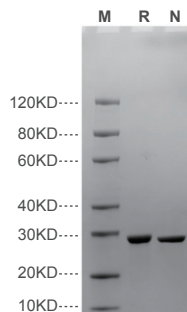
#### Broad applications:

- Digestion of contaminating nucleic acids
- Reduction of cell lysate viscosity
- Reduction of cell culture clumping
- Increase in pellet and supernatant separation
- Increase in particle purification yields by reducing aggregation
- Increased protein purification yields
- Sample preparation and electrophoresis for various applications including: ELISA, column chromatography, two-dimensional electrophoresis, blot analysis, etc.

# 1.3 Benz-Neburase™ purity

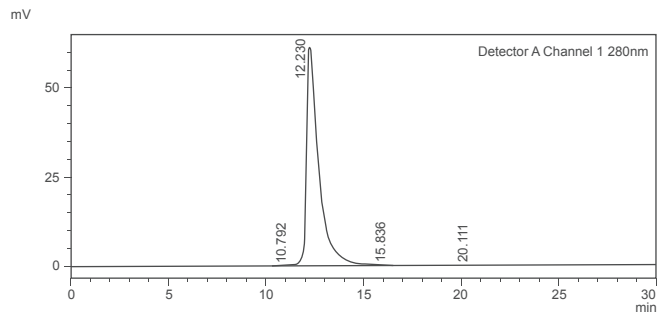
Overview

GenScript's Benz-Neburase is highly pure showing over 99% purity with SEC-HPLC analysis, with stringent quality control metrics.



**Figure 1:**

Analysis of the Benz-Neburase by SDS-PAGE showed  $\geq 95\%$  purity



**Figure 2:**

Analysis of the Benz-Neburase by SEC-HPLC showed  $\geq 99\%$  purity

## 1.4 Benz-Neburase™ quality controls

Overview

Quality Control Metrics	Standards
Appearance	Colorless, clear liquid
Specific Activity	$\geq 1.1 \times 10^8$ U/mg
Enzyme Activity	$\geq 250$ U/ $\mu$ l
Purity	$\geq 95\%$ by SDS-PAGE
	$\geq 99\%$ by SEC-HPLC
Endotoxin Level	$\leq 0.01$ EU/kU
Host Cell Protein	$\leq 10$ $\mu$ g/mg
Protease Activity	Non-detectable
Bioburden	<1 CFU/ml
Heavy Metal Residue	$\leq 10$ ppm
Mycoplasma	Negative

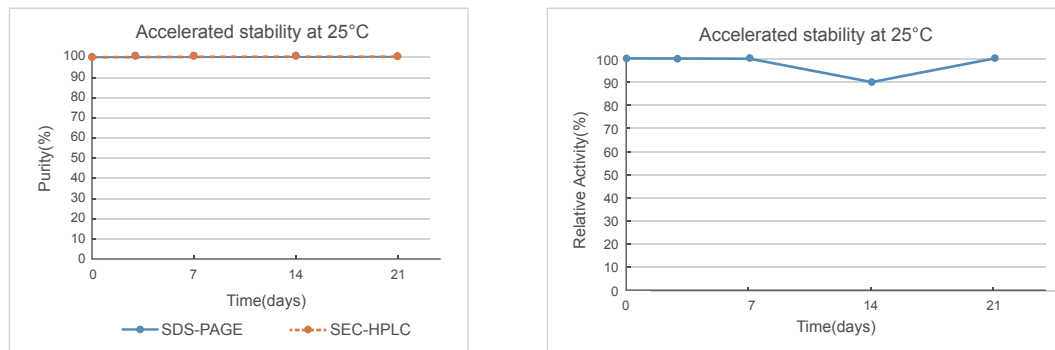
**Table 1: Quality control metrics for Benz-Neburase**

## 1.5 Benz-Neburase™ stability

### Overview

Product testing showed that GenScript's Benz-Neburase is stable at 25 °C storage

Note: To maintain optimum enzyme activity, we do not recommend storage at temperatures higher than -20 °C or repeated freeze/thaw cycles.



**Figure 3:**

The Benz-Neburase was stored at 25°C for 21 days. The purity (degradation) and activity of the nuclease were tested on day 0, 3, 7, 14 and 21 with storage at 25°C. PAGE and HPLC analysis indicate no degradation of the nuclease thus no loss of purity. The nuclease maintained relative activity greater than 90% throughout the 21 day period.

The test results indicate Benz-Neburase is highly stable.

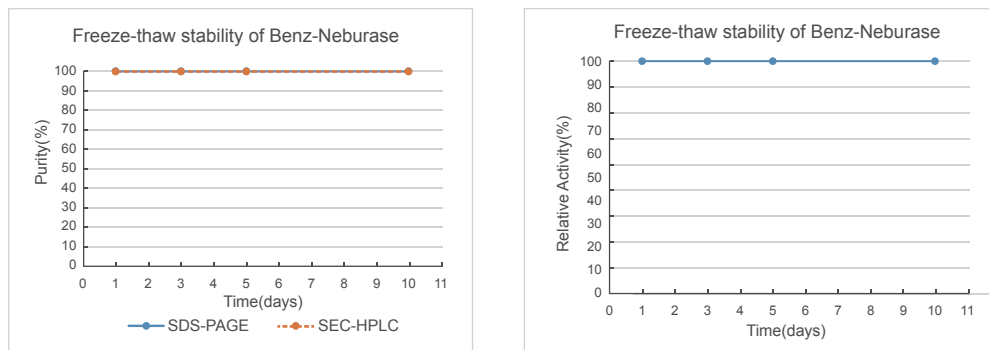


## 1.6 Benz-Neburase™ stability

### Overview

Product testing showed that GenScript's Benz-Neburase has minimal reduction in activity after four freeze-thaw cycles.

Note: To maintain optimum enzyme activity, we do not recommend storage at temperatures higher than -20 °C or repeated freeze/thaw cycles.



**Figure 4:**

The Benz-Neburase was frozen at -20°C and thawed 4 times followed by storage at -20°C. The first freeze-thaw cycle was performed on day 1, followed by the second, third and fourth freeze thaw cycles on day 3, day 5 and day 10, respectively. The purity (degradation) and activity of the nuclease were tested on day 1, 3, 5 and 10. PAGE and HPLC analysis indicate no degradation of the nuclease thus no loss of purity. The relative activity of the nuclease showed no reduction of activity through the four freeze-thaw cycles. The test results indicate Benz-Neburase is highly freeze-thaw stable.

## 2.1 Impact of reaction conditions on the performance of Benz-Neburase™

Reaction  
Conditions

GenScript's Benz-Neburase is highly effective under a wide range of conditions.

Condition	Optimal*	Effective**
Mg <sup>2+</sup>	1-2 mM	1-10 mM
pH	8.0-9.2	5.0-11.0
Temperature	37-45 °C	25-55 °C
Salt ions (Na <sup>+</sup> , K <sup>+</sup> , etc.)	0-20 mM	0-300 mM
PO <sub>4</sub> <sup>3-</sup>	0-10 mM	0-40 mM
Urea	4 M	0-6 M
SDS	SDS inactivates Benz-Neburase in 10 minutes at any concentration.	

Table 2: Reaction conditions for Benz-Neburase.

\*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.

## 2.2 Impact of reaction conditions on the performance of Benz-Neburase™

Reaction  
Conditions

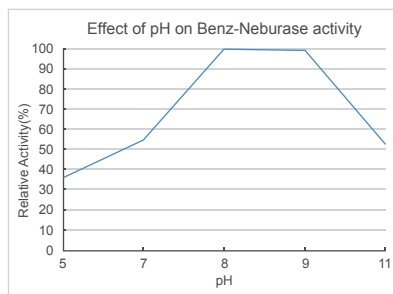


Figure 5: Effect of pH

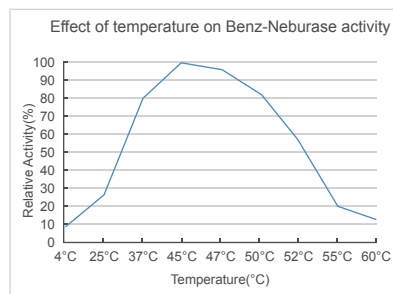


Figure 6: Effect of Temperature

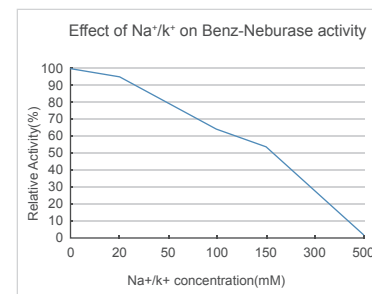


Figure 7: Effect of Ions

## 2.2 Impact of reaction conditions on the performance of Benz-Neburase™

Reaction  
Conditions

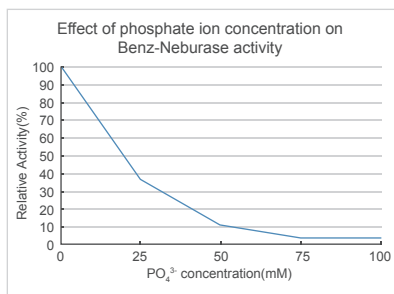


Figure 8: Effect of Phosphate

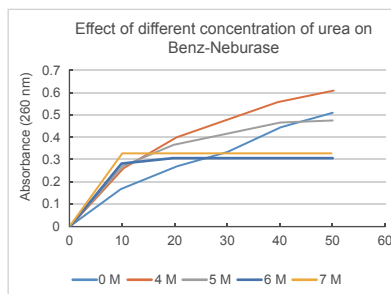


Figure 9: Effect of Urea

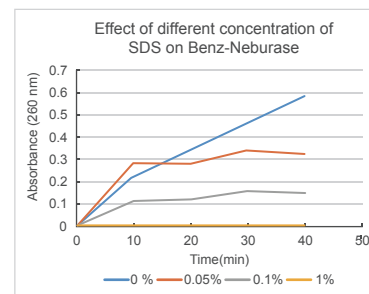
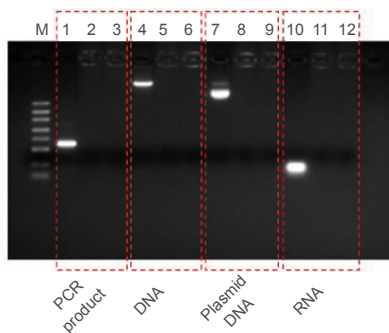


Figure 10: Effect of SDS

## 3.1 Digestion of various types of nucleic acids using Benz-Neburase™

### Applications



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

#### Application:

- Nucleic acid type: DNA (multi-form)/RNA
- Process flow: direct nuclease treatment
- Dosage: 1 unit /20  $\mu$ l
- Temperature: 37°C
- Reaction time: 30 min

In 20  $\mu$ l reaction volume, use 1U of Benz-Neburase to digest different kinds of nucleic acid at 37°C for 10 minutes.

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

## 3.2

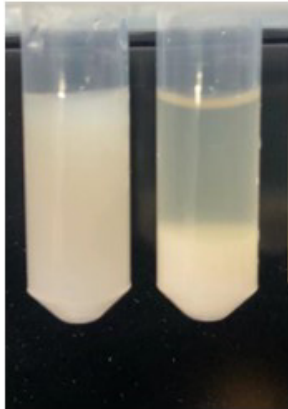
# Reduction of the viscosity of bacterial lysate using Benz-Neburase™

Applications

### Benz-Neburase™

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2.5



#### Application:

- Bacteria type: Escherichia coli
- Process flow: Centrifuge the bacteria, remove the supernatant, add lysate and nuclease
- Dosage: 2.5 U/ml
- Temperature: 37°C
- Reaction time: 30 min

**Step 1:** Centrifuge the bacterial culture, remove the supernatant, 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 minutes.

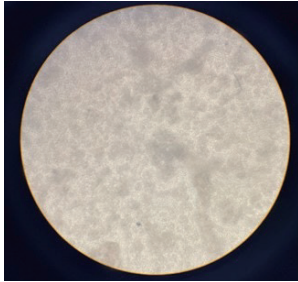
**Step 3:** Centrifuge to observe the viscosity of the precipitate and supernatant.

The test results show that Benz-Neburase can greatly reduce the viscosity of bacterial lysate.

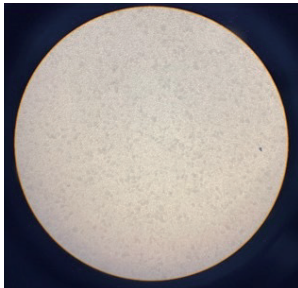
## 3.3 Prevention of cell clumping using Benz-Neburase™

### Applications

Clumped Cells



Cells Treated with Benz-Neburase



#### Application:

- Cell type: SUP-T1 cells and K562 cells
- Process flow: Spread the clumped cells in a plate, treat the cells with Benz-Neburase, observe the cells by a microscope
- Dosage: 50 units /ml
- Temperature: 37°C
- Reaction time: 30 minutes

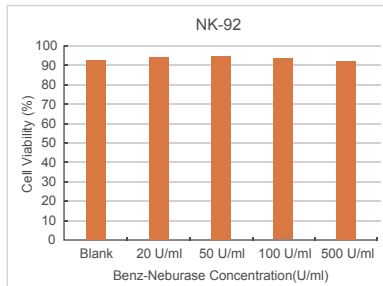
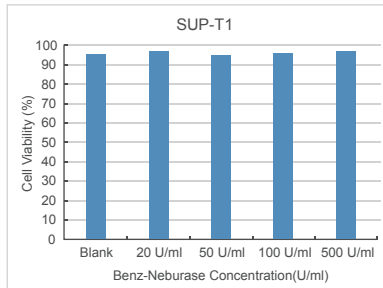
**Step 1:** Spread the adhered cells in a 24-well plate and treat them with control buffer (above) and 50 U/ml Benz-Neburase (below) at 37°C for 30 minutes.

**Step 2:** Observe the cells using a microscope.

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

## 3.3 Prevention of cell clumping using Benz-Neburase™

### Applications



#### Application:

- Cell type: SUP-T1 cells and NK-92 cells
- Process flow: Treat the cells with different Units of Benz-Neburase, culture the cells overnight, measure the cell viability
- Dosage: 20-500 units/ml
- Temperature: 37°C
- Reaction time: overnight

**Step 1:** Treat SUP-T1 cells (above) and NK-92 cells (below) with 2  $\mu$  l of Benz-Neburase at different concentrations (20-500 U/ml).

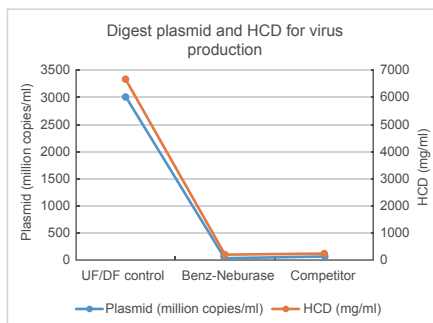
**Step 2:** incubate overnight in an incubator at 37°C in 5% CO<sub>2</sub>.

The test results indicate Benz-Neburase has minimal to no impact on the cell viability.



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

### Applications



#### Application:

- Expression system: HEK293
- Virus type: LV
- Process flow: adding nuclease to virus supernatant
- Purpose: Sample plasmid residue and host DNA residue
- Dosage: 20 units/ml
- Temperature: 37°C
- Reaction time: 1 h

**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.

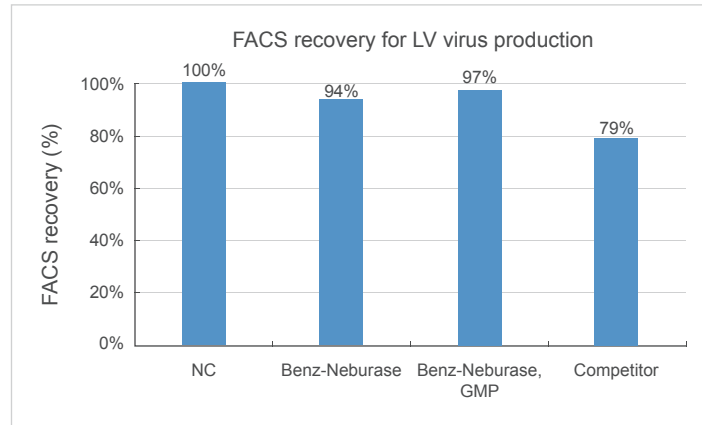
**Step 3:** After the incubation, centrifuge to remove the cells and cell debris at 1300 g for 10 min.

**Step 4:** After the centrifugation, measure the HCD and plasma residues in the samples.

The test results show that Benz-Neburase can remove DNA and plasmid residue in virus production process more effectively than the competitor product.

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

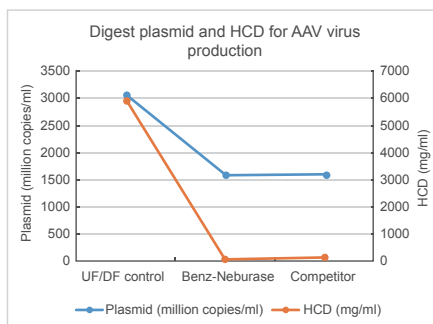
Applications



Fluorescence activated Cell Sorting (FACS) was used to determine the functional titer of lentiviral particles. The analysis data indicate that the use of Benz-Neburase has minimal impact on the viral production recovery. The data further indicates that the GenScript Benz-Neburase outperforms the competitor product.

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

### Applications



### Application:

- Expression system: HEK293
- Virus type: AAV
- Process flow: adding nuclease to virus supernatant
- Purpose: Remove sample plasmid residue and host DNA residue
- Dosage: 50 units/ml
- Temperature: 37°C
- Reaction time: 1 h

**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.

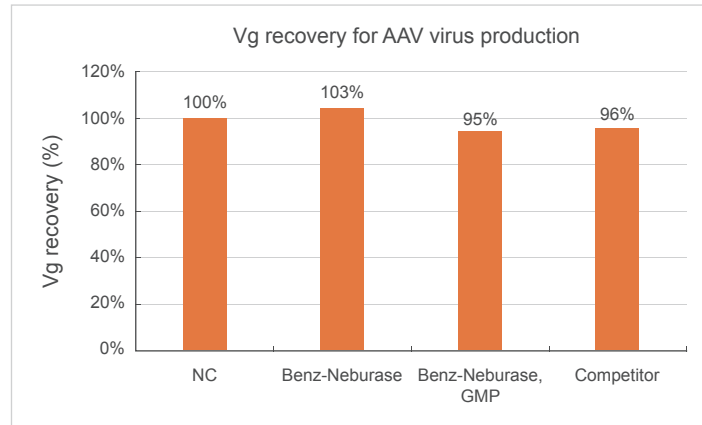
**Step 2:** After the incubation, centrifuge to remove the cells and cell debris at 1600 g for 10 min.

**Step 3:** After the centrifugation, measure the HCD and plasmid DNA residues in the samples.

The test results show that Benz-Neburase can also remove DNA and plasmid residue in AAV virus production process more effectively than the competitor product.

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

Applications



Viral genome (Vg) recovery was used to quantify the adeno associated viral production. The analysis data indicate that 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, while the Benz-Neburase GMP has comparative performance.

## 4.1 Ordering Information

### Ordering Information

Cat. No	Product name	Size
Z03626	Benz-Neburase™, His	10 kU; 100 kU; 500 kU
Z03627	Benz-Neburase™ GMP, His-tag	10 kU; 100 kU; 500 kU
Z03695	Benz-Neburase™, tag-free	10 kU; 100 kU; 500 kU
Z03708	Benz-Neburase™ GMP, tag-free	10 kU; 100 kU; 500 kU
L00886	Benz-Neburase™ ELISA Kit	48 T; 96 T

If you are interested in an alternative version of Benz-Neburase, contact us at [product@genscript.com](mailto:product@genscript.com)



**[www.GenScript.com](http://www.GenScript.com)**

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