Version: 01

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Benz-Neburase™ Nuclease ELISA Kit User Manual

Cat. No.： L00886

96 Tests

The users must read this technical manual carefully before starting the test.

For research use only. Not for use in diagnostic procedures.

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Contents

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I. Product Description

GenScript Benz-Neburase™ Nuclease ELISA Kit is a sensitive assay to detect and quantify residual endonuclease impurity in purification and manufacturing processes of viral vectors and vaccines where utilized Benz-Neburase™ Nuclease or other *Serratia marcescens* endonucleases. Benz-Neburase™ Nuclease ELISA Kit is based on sandwich ELISA with a detection limit of 8.40 pg/ml. It is a simple, accurate and reproducible solution for residual endonuclease quantitation to add to the purification process, quality control, and release testing.

Benz-Neburase™ Nuclease is genetically engineered endonuclease from *S. marcescens* that cleaves all forms of DNA and RNA non-specifically, including single stranded, double stranded, linear and circular. They are mostly used to remove residual nucleic acids during production process of biological molecules in biopharmaceutical manufacturing including but not limited to vaccine production, viral vector production, and manufacturing of gene and cell therapy-related products.

II. Assay Principle

Benz-Neburase™ Nuclease ELISA Kit is a sandwich ELISA that utilizes two different monoclonal antibodies binding two different epitopes of Benz-Neburase™ Nuclease.

When Benz-Neburase™ Standard or testing samples are added to the Capture Plate, Benz-Neburase™ Nuclease in the Standard or testing samples can be captured by the Anti- Benz-Neburase™ Nuclease antibody which is coated on the Capture Plate. Then the Detection Reagent (containing Anti- Benz-Neburase™ Nuclease antibody conjugated with biotin) is added to interact with the Benz-Neburase™ Nuclease bound on the plate. Enzyme Conjugate (Streptavidin-Horseradish Peroxidase conjugate) is added to interact with the biotin conjugated anti- Benz-Neburase™ Nuclease antibody. After the washing steps, TMB Solution (3, 3', 5, 5'-Tetramethylbenzidine solution) is added resulting in formation of blue color. The reaction is stopped by adding Stop Solution. Application of the Stop Solution results in the color changing from blue to yellow. The intensity of the color (Optical density, OD) can be measured at 450 nm and 650 nm by a microplate reader. The amount of Benz-Neburase™ Nuclease in the sample is positively proportional with the OD value and can be calculated using a standard curve generated using the Benz-Neburase™ Standard.

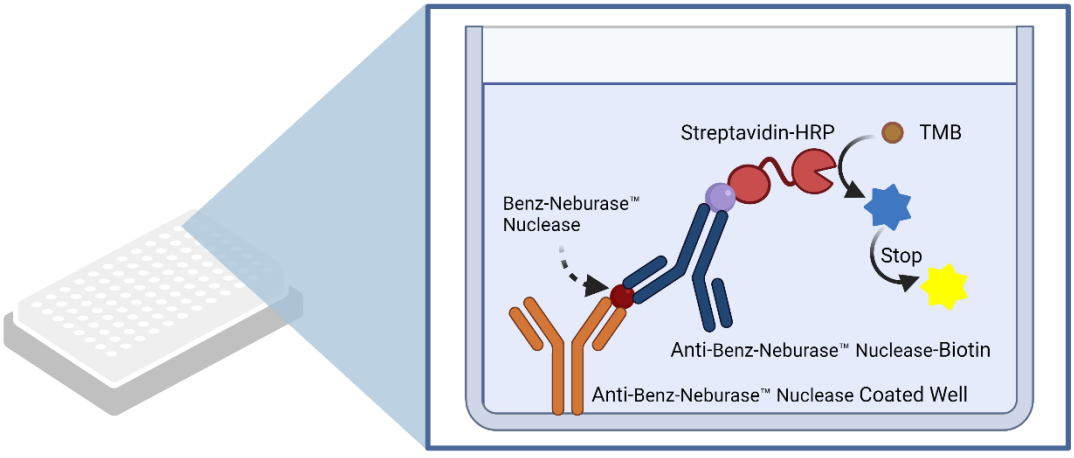

Figure 1: Schematic diagram of Benz-Neburase™ Nuclease ELISA Kit

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III. Key Features

Features	Specifications
Detection Range	15.63 pg/ml-1000 pg/ml
Limit of Detection (LOD)	8.40 pg/ml
Sample Types	Mammalian and bacterial cell lysates, cell culture supernatant
Specificity	This kit is validated to recognize Benz-Neburase™ GMP, His-tag (Cat. No. Z03627, GenScript), Benz-Neburase™ GMP, tag free (Cat. No. Z03708, GenScript), and *S. marcescens* nucleases from other suppliers.

IV. Kit Contents

The kit provides the following reagents and solutions.

Component	Quantity	Part. No.
Capture Plate	8 wells × 12 strips, 1 plate	L00886 - 80
Benz-Neburase™ Standard (100 ng/ml)	100 µl × 1 vial	L00886 - 10
100 × Detection Reagent	120 µl × 1 vial	L00886 - 20
100 × Enzyme Conjugate	120 µl × 1 vial	L00886 - 30
Sample Dilution buffer	50 ml × 1 vial	L00886 - 60
Assay Buffer A	12 ml × 1 vial	L00886 -21
Assay Buffer B	12 ml × 1 vial	L00886 - 31
20 × Wash Solution	40 ml × 1 vial	L00886 - 70
TMB Solution	12 ml × 1 vial	L00886 - 40
Stop Solution	6 ml × 1 vial	L00886 - 50
Plate Sealer	2 pieces	N/A
User Manual	1 copy	N/A

- Capture Plate: Plate sealed in a foil pouch with a desiccant.
- Benz-Neburase™ Standard: Contains 100 ng/ml of Benz-Neburase™ GMP, tag free (Cat. No. Z03708, GenScript). The Benz-Neburase™ Standard must be diluted to generate the standard curve.

V. Storage

The unopened kit is stable for at least 12 months from the date of manufacture if stored at 2 °C to 8 °C, and the opened kit is stable for up to 1 month from the date of opening at 2 °C to 8 °C. **Do not freeze the reagents in this kit.**

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VI. Reagents/Equipment Required But Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm and 650 nm
- Automated microplate washer
- Distilled water
- Graduated cylinder
- Conical tubes
- Centrifuge tubes
- Pipettes capable of pipetting 10 µl, 100 µl, 200 µl and 1000 µl
- Pipette tips of 10 µl, 100 µl, 200 µl and 1000 µl
- Multichannel pipette
- Disposable reagent reservoirs
- Paper towel
- Timer
- Refrigerator
- 25 °C incubator
- Centrifuge

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VII. Protocol

i. Before Start

1. Sample Preparation

Note:

- *Insoluble particulates or precipitates should not be present in testing samples. An additional filtration or centrifugation is recommended to remove any insoluble particles or precipitates in the samples.*
- *pH of testing samples shall be adjusted to in between pH 6.8 and pH 7.4.*

1) Cell Culture Supernatant

Use fresh cell culture supernatant for test. Centrifuge samples to remove any particulates and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

2) Cell Lysate

Generally, a commercial cell lysis buffer can be used for cell lysate preparation according to the vendors' instructions. After lysis, centrifuge samples to remove any cell debris and other particulates, collect the supernatant for test or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

2. Reagent Preparation

1 × Wash Solution

Dilute 20 × Wash Solution at 1: 19 v/v with distilled water. For example, dilute 10 ml of 20 × Wash Solution with 190 ml of distilled water to make 200 ml of 1 × Wash Solution. The 1 × Wash Solution can be stored at 2-8 °C.

1 × Detection Reagent

Dilute 100 × Detection Reagent at 1: 99 v/v with Assay Buffer A.

1 × Enzyme Conjugate

Dilute 100 × Enzyme Conjugate at 1: 99 v/v with Assay Buffer B. ***Store in a cool, dark place in a tightly closed container.***

Benz-Neburase™ Standard Working Solutions

Prepare serial dilution as described in the following steps：

Pipette	Into	Standard Concentration
10 µl Benz-Neburase™ Standard (100 ng/ml)	990 µl Sample Dilution Buffer，and mix	1000 pg/ml
500 µl 1000 pg/ml Standard	500 µl Sample Dilution Buffer，and mix	500 pg/ml
500 µl 500 pg/ml Standard	500 µl Sample Dilution Buffer，and mix	250 pg/ml
500 µl 250 pg/ml Standard	500 µl Sample Dilution Buffer，and mix	125 pg/ml
500 µl 125 pg/ml Standard	500 µl Sample Dilution Buffer，and mix	62.5 pg/ml
500 µl 62.5pg/ml Standard	500 µl Sample Dilution Buffer，and mix	31.25 pg/ml
500 µl 31.25 pg/ml Standard	500 µl Sample Dilution Buffer，and mix	15.63 pg/ml
500 µl Sample Dilution Buffer	/	0 pg/ml

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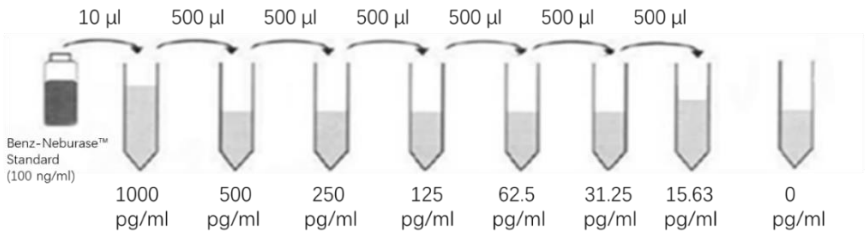

3. Capture Plate Preparation

Prepare the strips as needed for the assay, return unused strips back into the foil pouch and reseal it to prevent moisture, and store at 2 - 8 °C.

Note:

- *It is recommended that all standards and samples are prepared and tested in duplicate.*
- *Make sure the strips are tightly snapped in the plate frame.*

ii. Assay Procedure

Note:

- *All reagents in the kit and samples should be equilibrated to room temperature before use.*
- *Preliminary experiments are suggested to determine optimal sample dilutions. Dilute samples with the Sample Dilution Buffer provided in the kits.*
- *The assays should be performed at room temperature (25 ± 2 °C).*

1. Standards and Samples Incubation

1) Add 100 µl of Benz-Neburase™ Standard Working Solution or samples to each well of microplate.
2) Cover the plate with a Plate Sealer and incubate at room temperature (25 ± 2 °C) for 60 minutes.
3) After the incubation, remove the sealer and wash the plate with 250 µl of 1 × Wash Solution five times.
    - Manual wash: Add 250 µl of 1 × Wash Solution to each well, soak for about 30 seconds and then remove the liquid completely, repeat the wash step for additional 4 times.
    - Automated wash: The wash steps can be also completed by an automated washer, perform the wash steps referring to supplier's protocol and instructions.
4) Pat the plate against clean paper towels to completely remove residual liquid in the wells.

2. Detection Antibody Incubation

5) Add 100 µl of 1 × Detection Reagent to each well.
6) Cover the plate with a Plate Sealer and incubate at room temperature (25 ± 2 °C) for 60 minutes.
7) After the incubation, remove the sealer and wash the plate with 250 µl of 1 × Wash Solution five times as in step 3.
8) Pat the plate against clean paper towels to completely remove residual liquid in the wells.

3. Enzyme Conjugate Incubation

9) Add 100 µl of 1 × Enzyme Conjugate to all wells.
10) Cover the plate with a Plate Sealer and incubate at room temperature (25 ± 2 °C) for 15 minutes.

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11) After the incubation, remove the sealer and wash the plate with 250 µl of 1 × Wash Solution five times as in step 3.
12) Pat the plate against clean paper towels to completely remove residual liquid in the wells.

4. Substrate Reaction and Absorbance Measurement

13) Add 100 µl of TMB Solution to all wells.
14) Cover the plate with a Plate Sealer and incubate at room temperature for 15 minutes. Protect from light.
15) Add 50 µl of Stop Solution to each well to stop the enzyme reaction.
    Note: *To ensure test stability, it's recommended to read the plate immediately.*
16) Read absorbance of the plate on a microplate reader at 450 nm and 650 nm, respectively. Calculate the final optical density (OD) according to the equation: $OD=OD450_{nm} - OD650_{nm}$.

5. Results Analysis

17) Average the duplicate readings for each standard, control, and sample. Calculate the corrected OD by subtracting the average OD value of 0 pg/ml standard.
18) Generate a standard curve by plotting the average corrected OD for each standard on the vertical (Y) axis versus the standard concentration on the horizontal (X) axis in a linear fitting model.
19) The concentration of Benz-Neburase™ Nucleases or other *S. marcescens* nucleases in a sample can be calculated according to the standard curve. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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VIII. Summary of Assay Procedure

1. • Add 100 µl of Benz-Neburase™ Standard Working Solution or sample to each well. Incubate at 25 °C for 60 minutes.
2. • Wash plate with 250 µl of 1 × Wash Solution five times.
3. • Add 100 µl of 1 × Detection Reagent to each well. Incubate at 25 °C for 60 minutes.
4. • Wash plate with 250 µl of 1 × Wash Solution five times.
5. • Add 100 µl of 1 x Enzyme Conjugate to each well. Incubate at 25 °C for 15 minutes.
6. • Wash plate with 250 µl of 1 × Wash Solution five times.
7. • Add 100 µl of TMB Solution to each well. Incubate the plate in dark at 25 °C for 15-20 minutes.
8. • Add 50 µl of Stop Solution to each well to stop the reaction.
9. • Read absorbance of the plate on a microplate reader at 450 nm and 650 nm immediately.

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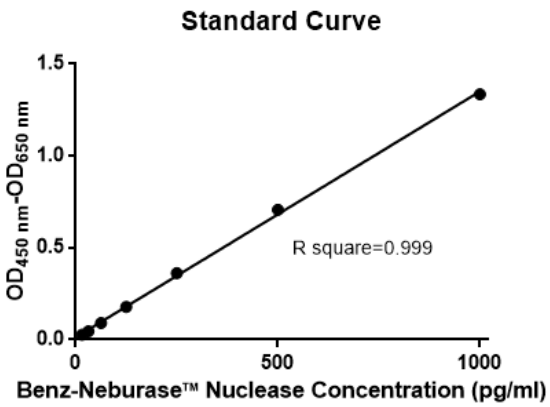
IX. Typical Assay Data

The standard curve is provided for demonstration only. It should be generated for each set of samples assayed.



Standards (pg/ml)	$OD_{450\ nm}$-$OD_{650\ nm}$			Corrected
	Duplicate 1	Duplicate 2	Average	
1000	1.338	1.377	1.358	1.336
500	0.730	0.728	0.729	0.707
250	0.389	0.379	0.384	0.362
125	0.198	0.202	0.200	0.178
62.5	0.115	0.110	0.113	0.091
31.25	0.067	0.067	0.067	0.045
15.63	0.047	0.050	0.049	0.027
0	0.022	0.022	0.022	/

i. Precision

Intra-Assay Precision

Three samples of known concentration were tested 20 times on the same plate to evaluate intra-assay precision of the kit.

Inter-Assay Precision

Three samples of known concentration were tested in 36 separate assays to evaluate inter-assay precision of the kit. Assays were performed by at least three technicians using two lots of components.

	Intra-assay			Inter-assay		
Sample	1	2	3	1	2	3
n	20	20	20	36	36	36
Mean (pg/ml)	558.02	280.19	135.52	578.00	288.47	139.62
Standard Deviation	13.77	8.84	8.04	29.73	12.81	8.77
CV (%)	2	4	6	5	4	6

ii. Sensitivity

The limit of detection (LOD) of Benz-Neburase™ Nuclease ELISA Kit is 8.40 pg/ml.

LOD is defined as the ratio of three standard deviations of twenty zero standard replicates divided by the slope of the standard curve according to IUPAC.

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iii. Recovery

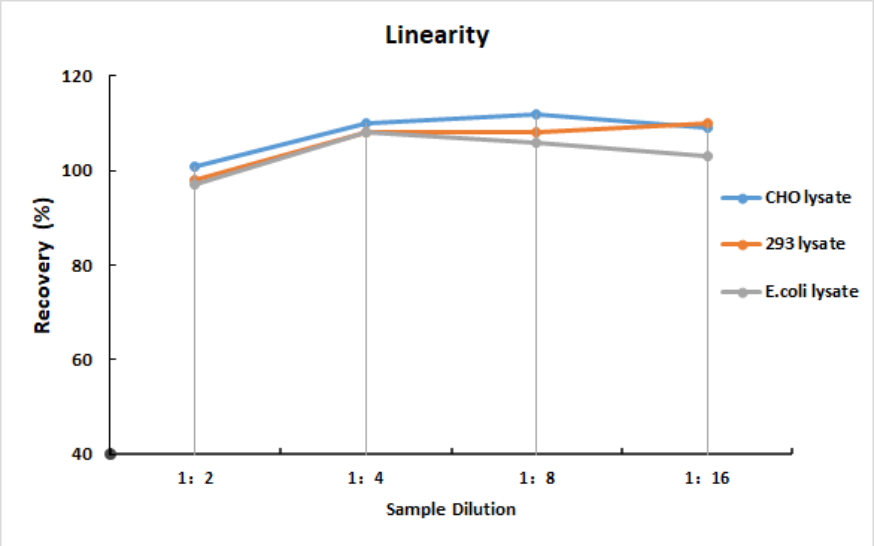
The recovery of Benz-Nebulase™ nuclease spiked to different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Recovery Average (%)	Recovery Range (%)
CHO lysate (n=6)	97	92-101
HEK 293 lysate (n=6)	95	90-101
E. coli lysate (n=6)	93	86-100

iv. Linearity

To assess the linearity of the assay, high known concentrations of Benz-Nebulase™ Nuclease were spiked into various samples and then were diluted with Sample Dilution Buffer to produce samples with values within the dynamic range of the assay.

Dilution		*CHO* lysate (n=2)	*HEK 293* lysate (n=2)	*E. coli* lysate (n=2)
1:2	Average (%)	101	98	97
	Range (%)	100-101	95-101	96-97
1:4	Average (%)	110	108	108
	Range (%)	106-114	107-108	107-108
1:8	Average (%)	112	108	106
	Range (%)	111-113	102-114	101-110
1:16	Average (%)	109	110	103
	Range (%)	109-109	109-111	97-109



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v. Specificity

To assess the specificity of this kit, Benz-Neburase™ GMP, His-tag (Cat. No. Z03627, GenScript), Benz-Neburase™ GMP, tag-free (Cat. No. Z03708, GenScript), and a competing product from another supplier was spiked to different levels in samples throughout the range of the assay and the recovery of each product were evaluated.

Samples (n=6)		High Concentration (1000 pg/ml)	Low Concentration (62.5 pg/ml)
Benz-Neburase™ GMP, His-tag	Average (%)	99	95
	Range (%)	95-105	89-101
Benz-Neburase™ GMP, tag-free	Average (%)	89	91
	Range (%)	88-89	90-92
Competing product A	Average (%)	114	113
	Range (%)	112-115	96-124

The data indicates that GenScript's Benz-Neburase™ ELISA Kit (Cat. No. L00886, GenScript) is able to detect Benz-Neburase™ GMP, His-tag (Cat. No. Z03627, GenScript) and Benz-Neburase™ GMP, tag-free (Cat. No. Z03708, GenScript) manufactured by GenScript, and the same endonuclease manufactured by other suppliers.

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X. Troubleshooting

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Poor Standard Curve	Improper preparation of standards	Prepare new standards as the manual describes
	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or sources	Never substitute any components from another kit
	Components are not brought to room temperature prior to assay	Repeat assay with components that have been equilibrated to room temperature
	Incubation steps are performed at wrong temperatures	Perform incubation step as the manual describes
Weak/No Signal	TMB substrate is not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	TMB substrate is contaminated	Use new TMB substrate
	Did not add the proper volumes of reagents	Repeat assay with the required volumes in manual
	Did not incubate the plate for proper time or temperature	Follow the manual to repeat assay
	Did not read the plate immediately after stop solution was added	Read the plate within 2 minutes after adding stop solution
High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	TMB substrate is contaminated	Use new TMB substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
	TMB substrate is exposed to light	Use new TMB substrate

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XI. Related Products

Cat. No	Product name	Size
Z03626	Benz-Neburase™, His-tag	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

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XII. Plate Layout

Use this plate layout to record standards and samples assayed.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

For research use only. Not for use in diagnostic procedures.