

# BenzoNuclease<sup>®</sup>, GMP Grade

Cat No.: GMP-1707      animal-free, ampicillin-free

## 01/ Product Description

BenzoNuclease<sup>®</sup> is a recombinant endonuclease derived from *Serratia marcescens*. The protein is expressed in *Escherichia coli* (*E. coli*). BenzoNuclease<sup>®</sup> has been shown to exert a broad spectrum of substrate specificity to degrade both DNA and RNA—whether single-stranded, double-stranded, linear, circular or supercoiled. No base preference is observed. As with all endonucleases, BenzoNuclease<sup>®</sup> hydrolyzes internal phosphodiester bonds present between the nucleotides. Upon complete digestion, all free nucleic acids present in solution are reduced to 5'-monophosphate-terminated oligonucleotides, which are three to five bases in length.

In scientific research, BenzoNuclease<sup>®</sup> can be used as the preferred enzyme preparation for supernatant of cultured cells and viscosity removal of cell lysate, which can remove nucleic acid interference and improve the subsequent protein purification or functional research. At the same time in the vaccine industry, protein and polysaccharide pharmaceutical industry, used to remove the host residual nucleic acid, greatly reduce the vaccine and protein products of nucleic acid pollution, reduce the processing steps, improve the yield of products.

This product is expressed by large-scale fermentation of *E. coli*, and is produced with raw materials of medicinal specifications. The host protein residue, nucleic acid residue and common pathogens are strictly controlled, and the production and quality management procedures of the product comply with GMP regulations to ensure the traceability of the production process and all raw materials.

## FDA DMF



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Application Type/Number: MF035864  
eCTD Sequence Number: 0001

## 02/ Application

- Used to remove exogenous nucleic acid from vaccine products, reduce the risk of residual toxicity of nucleic acid and improve product safety.
- Used to reduce the viscosity of feed liquid caused by nucleic acid, shorten the processing time and increase the protein yield.
- Used to remove nucleic acid winding on the surface of particles (viruses, inclusion bodies, etc.), which is conducive to the release and purification of particles.
- Used to prepare samples for ELISA, column chromatography, 2D electrophoresis and Western blot analysis. The resolution and recovery can be improved after BenzoNuclease<sup>®</sup> treatment.
- Used to prevent cell clumping.

### 03/ Quality Criterion

Element	Standard
Appearance	clear, transparent solution
Visible Particles	meet the specification
pH	7.5-8.5
Activity	250-400 U/μl
Spec. Activity	$\geq 1.1 \times 10^6$ U/mg
Purity	$\geq 99\%$
Protease Activity	no protease activity detectable
Bacterial Endotoxins	$< 0.01$ EU/KU
Host-cell Protein Residues	$\leq 0.005\%$
Sterility	meet the specification
Mycoplasma	negative
Heavy Metal Residue	$\leq 10$ ppm

### 04/ Complying to Following Regulations

- ISO 9001:2015, certified facility.
- GMP Appendix - Cell Therapy Products, National Medical Products Administration.
- General Theory of Gene Therapy for Human Use, Chinese Pharmacopoeia Commission.
- USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
- USP Chapter <92>, Growth Factors and Cytokines Used in Cell Therapy Manufacturing.
- Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

### 05/ BenzoNuclease® at a glance

Product Name	BenzoNuclease®, GMP Grade
Pack Size	100KU/200KU/5000KU
Molecular Weight	32kDa
pI	6.99
Tag	6×His
Purity	$\geq 99\%$ (SDS-PAGE, SEC-HPLC)
Optimal pH	8
Optimal Temperature	37°C
Cofactor	1-10 mM Mg <sup>2+</sup>
Formulation	20 mM Tris-HCl pH8.0, 2 mM MgCl <sub>2</sub> , 20 mM NaCl, 50% (v/v) glycerol
Storage Condition	Store at -20±5°C, Avoid repeated freeze-thaw cycles. Properly stored BenzoNuclease® is stable for at least 24 months.
Unit Definition	In a 2.625 mL reaction system at 37°C and pH 8.0, one unit of BenzoNuclease® is defined as the amount of enzyme that causes a change in absorbance at 260 nm of 1.0 absorption units within 30 minutes.

### 06/ Product Packaging

SKU	Size	Item	Quantity
GMP-1707-100KU	100KU	BenzoNuclease®, GMP Grade (250U/μl)	400μl
GMP-1707-200KU	200KU	BenzoNuclease®, GMP Grade (250U/μl)	800μl
GMP-1707-5000KU	5000KU	BenzoNuclease®, GMP Grade (250U/μl)	20ml

## 07/ Reaction Condition

Condition	Optimal*	Effective**
Mg <sup>2+</sup>	1-2mM	1-10mM
pH	8.0-9.2	6.0-10.0
Temperature	37°C	0-42°C
DTT	0-100mM	>100mM
BME	0-100mM	>100mM
Na <sup>+</sup> , K <sup>+</sup> , etc.	0-20mM	0-150mM
PO <sub>4</sub> <sup>3-</sup>	0-10mM	0-100mM

\*: Optimal is defined as the condition under which BenzoNuclease® retains >90% of its activity.

\*\*: Effective is defined as the condition under which BenzoNuclease® retains >15% of its activity.

## 08/ Reference Dosage

Usage scenarios	Preparation of electrophoretic protein samples	Protein production	Production of vaccines and viruses	Cell medicine
Number of cells	1×10 <sup>6</sup> cells (10mg tissue)	1g wet weight (10ml resuspension)	1L fermentation supernatant	1L cultures
Minimum dosage	125 U	250 U	100 U	100U
Recommended dosage	500 U	2500 U	25000 U	5000U
Time	Normally the action time is 37°C for 15~60 min, 25°C for 30~120 min			

## 09/ Note

- For sample repackaging, please use sterile pyrogen-free bottles with medical device certificates. Generally, Eppendorf tubes on the market are not medical devices.
- The formulations of Novoprotein products have been optimized, and generally samples stored at 4°C for two weeks do not affect their biological activity, but long-term storage at 4°C is not recommended.

## 10/ Q&A

- At what step do we add BenzoNuclease®?  
It depends on the purpose for which you use BenzoNuclease®. For example, BenzoNuclease® can be added to the sample along with a lysate reagent in the purification of proteins expressed in *E. coli*.
- When the reaction temperature is lower than 37°C, how to ensure the digestion effect of BenzoNuclease®?  
In the case of a fixed system, the digestion effect of BenzoNuclease® mainly depends on the amount of enzyme, reaction temperature and reaction time. When the reaction temperature is low, it is more recommended to extend the reaction time to ensure the digestion effect of BenzoNuclease® in order to avoid residual problems caused by excessive BenzoNuclease®.
- How do I inhibit BenzoNuclease® activity?  
There are process additives/agents that affect BenzoNuclease® activity—for example, it can be inhibited by high salts, like > 300 mM monovalent cations, > 100 mM phosphate, 100 mM ammonium sulfate, > 100 mM guanidine HCl. Other known inhibitors are chelating agents, like EDTA, which could cause loss of free Mg<sup>2+</sup>-ions (EDTA concentrations > 1 mM have shown to inhibit the enzymatic reaction). This can be reversed by adding more MgCl<sub>2</sub>.
- BenzoNuclease® is compatible with protease inhibitor cocktails?  
Yes. However, caution should be exercised, since many protease inhibitor cocktails include EDTA.

## 11/ Relative Product

Cat. No.	Product Name	Cat. No.	Product Name
PA018	BenzoNuclease® ELISA Kit	GMP-1709	BenzoNuclease® (Tag-free), GMP Grade