Salt Active Universal Nuclease (GMP Grade)

Please read the manual carefully before use.

Cat. No. LN301

Version No. Version 1.0

Storage: at -18°C or below for two years

Concentration: 25 units/μl

Description

Universal Nuclease, a genetically engineered endonuclease from Photobacterium Profundum, is a kind of no-specific endonuclease. The enzyme is produced and purified from Escherichia coli. This enzyme cleaves the phosphodiester bonds in the nucleic acid chains, degrading long-strand nucleic acids into short oligonucleotides with a length of 2–5 bases, with a 5′-phosphate group and a 3′-hydroxyl group. Therefore, this product efficiently degrades all kinds of DNA and RNA(double stranded, single stranded, linear or circular). It exhibits optimal activity at high salt concentrations (500 mM NaCl or KCl).

Kit Contents

Component	LN301-01 (2.5 KU)	LN301-02 (25 KU)
Salt Active Universal Nuclease	100 μl	1000 μl

Definition of Enzyme Activity

At 37°C, in a reaction system containing 25 mM Tris-HCl (pH 8.0 at 25°C), 500 mM NaCl and 5 mM $MgCl_2$, 1 unit (U) of Salt Active Universal Nuclease digests 50 μg/mL calf thymus DNA (Sigma, D-1501) within 30 minutes, producing an OD_{260} absorbance change of 1.0.

Enzyme Storage Buffer

25 mM Tris-HCl, 500 mM NaCl, 5 mM $MgCl_2$,50% Glycerol, pH 7.5, @25°C

Recommended Usage Conditions

This product maintains high stability and enzyme activity across a wide range of salt concentrations, temperatures, pH levels, and Mg^{2+} concentrations. Below are the recommended usage conditions for this product.

Condition Parameter	Optimum Condition	Valid Condition
NaCl/KCl	350-750 mM	0-1000 mM
Temperature	10-45°C	0-50°C
pH	7.7-9.5	> 6.5
Mg^{2+}	1-50 mM	-

*Note: Salt Active Universal Nuclease activity is ≥80% under the optimum conditions listed in this table, and the enzyme activity is ≥20% under valid conditions.

In addition, this product is tolerant with various reducing agents, surfactants, protein denaturants, and other common reagents. Below are some of the tolerance parameters of this product:

Condition Parameter	Optimum Condition	Valid Condition
Glycerol	0-10%	0-30%
PO_4^{3-}	0-70 mM	0-170 mM
EDTA	0-2 mM	0-5 mM
NH_4Cl	50-180 mM	0-300 mM
$(NH_4)_2SO_4$	0-120 mM	0-250 mM
Imidazole	0-100 mM	0-500 mM
Urea	0-2 M	0-4 M
Guanidine hydrochloride	0-100 mM	0-250 mM
SDS	0.002%	0-0.003%
Triton X-100	0-3.5%	0-15%
Tween 20	0-3%	0-10%
DTT	0-1 mM	0-10 mM
TCEP	0.1 mM	0-5 mM
PMSF	0-1 mM	-
Protease Inhibitor	≤2 mM EDTA	-

Reference Reaction System

To remove nucleic acids from cell extracts or lysates, the recommended dosage of this product depends on multiple factors, including experimental purpose, cell type, lysate composition, NaCl concentration, Mg^{2+} concentration, lysate pH, nucleic acid concentration, and reaction temperature.

For samples with a buffer composition of 25 mM Tris-HCl, 500 mM NaCl, 5 mM $MgCl_2$, pH 8.0, the recommended reaction system is provided in the table below:

Objective of the experiment	Sample type	Recommended enzyme amount (U/ml)	Reaction conditions
Nucleic acid removal	Protein solution	100	25-37°C for 30 min
	Reagent	100	25-37°C for 30 min
	Cell lysate	1000	25-37°C for 60 min or 4°C for overnight
	Supernatant fraction	500	25-37°C for 60 min or 4°C for overnight
Reducing viscosity	Cell lysate	25-50	25°C for 10-20 min

*Note:

Increase the enzyme amount or extend incubation time under the following conditions:

1. pH < 8.0, NaCl concentration higher or lower than 500 mM, reaction temperature below 25°C.
2. Reduce the enzyme amount or shorten incubation time if the reaction temperature is above 25°C.

Recommended Protocol

1. Nucleic Acid Removal

(1) Add $MgCl_2$ to adjust the Mg^{2+} concentration in the reaction system to 1–15 mM. Maintain the pH within 7.7–8.5.

(2) Add the recommended amount of this product (as specified in the table above) to initiate the reaction. Alternatively, adjust reaction conditions (e.g., temperature, incubation time) and enzyme dosage within an appropriate range based on experimental needs.

2. Enzyme Inactivation

Inactivate the product by adding reducing agents (TCEP or DTT). For different workflows, inactivation can be achieved by modulating temperature, incubation time, or reducing agent concentration. A temperature range of 25-37°C and incubation time of 5-10 minutes are generally recommended (achieving >99% enzyme inactivation). Refer to the table below for detailed inactivation parameters:

Inactivation conditions (temperature/time)	DTT	TCEP
4°C/18 hr	/	10 mM
25°C/60 min	10 mM	5 mM
30°C/30 min	10 mM	5 mM
40°C/30 min	5 mM	1 mM
50-70°C/30 min	1 mM	1 mM

Applications

1. For protein purification or protein extraction from tissue cell samples, this product is used to remove nucleic acid contamination and reduce sample viscosity.
2. Add Salt Active Universal Nuclease to cell or bacterial lysate to remove nucleic acids from crude extracts, reduce solution viscosity.
3. Remove the effect of negatively charged nucleic acids on two-dimensional SDS-PAGE protein samples, improve protein separation and enhance two-dimensional electrophoresis resolution.
4. Applications in pathogenic microbe diagnostics, such as host nucleic acid removal from metagenomic next-generation sequencing (mNGS) samples.
5. This product is used in vaccine production, virus purification, protein and polysaccharide pharmaceutical industry as a host residual nucleic acid removal reagent, reducing host nucleic acid residue to pg level and improving the efficacy and safety of biological products.
6. Other applications for host nucleic acid removal

Notes

- If the solution is acidic, or contains high concentrations of surfactants and protein denaturants, the amount of enzyme should be appropriately increased or the incubation time should be prolonged;
- If the sample is a crude product containing a large amount of protein, cell wall or other salts, the enzyme activity will be significantly inhibited, so the amount of enzyme should also be increased;
- The activity of the Salt Active Universal Nuclease is affected by factors such as ionic concentration, reaction temperature, and pH. For initial use, optimization of the enzyme amount is recommended.

FAQs & Solutions

1. What are the benefits of performing experiments under high-salt conditions?
   High-salt conditions (≥200 mM NaCl) disrupt electrostatic interactions between molecules, facilitating the separation of nucleic acids from proteins or viruses. Additionally, high salt concentrations reduce protein aggregation caused by electrostatic interactions. For these reasons, many biological assays require high-salt conditions. Salt Active Universal Nuclease can be directly used in such buffers to reduce viscosity and remove nucleic acids.
2. Can this product be used under low-salt conditions?
   Yes. Salt Active Universal Nuclease retains 60% activity at 250 mM NaCl or KCl and 15% activity at 0 mM NaCl or KCl. For low-salt applications, we recommend increasing enzyme dosage and/or extending incubation time based on experimental needs.

3. How to ensure efficient digestion when the reaction temperature is below 25°C?
   Digestion efficiency depends on enzyme amount, temperature, and incubation time. At lower temperatures (<25°C), we recommend prolonging the reaction time rather than increasing nuclease concentration, as excessive enzyme may lead to residual nuclease contamination.
4. How to remove this product from the sample?
   Due to its high pI value, the nuclease binds strongly to cation-exchange columns. Standard cation-exchange chromatography can effectively remove it.

Quality control

Key points	Standard
Physical Appearance	Colorless and transparent
Molecular Weight	30 kDa
Isoelectric Point (pI)	9.2
Purity	≥95% (SDS-PAGE)
Enzyme Activity	25 U/μl
Specific Activity	$\geq 2.0\times10^5$ U/mg protein
Cofactor Requirement	1~15 mM Mg^{2+}
Optimal pH Range	Optimal pH: 8.0 (functional range: 6.5-9.5)
Optimal Temperature Rang	Optimal temperature: 37°C (functional working range: 0-50°C)
Protease Activity	Not detected

For research use only, not for clinical diagnosis.

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