Universal Nuclease (GMP Grade)

Please read the datasheet carefully prior to use.

Cat. No. LN201

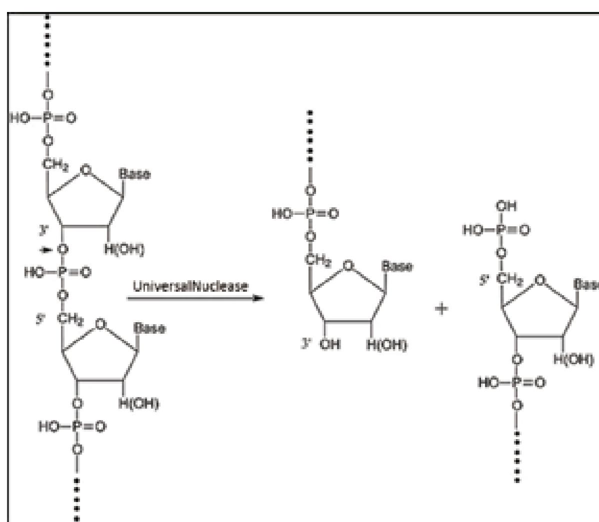
Storage: at -20°C for two years

Concentration: 250 units/μl

Description

Universal Nuclease, a genetically engineered endonuclease from Serratia marcescens, is a kind of no-specific endonuclease. The enzyme is produced and purified from Escherichia coli. It can digest nucleic acids to 2 to 5 bases 5' monophosphate terminated oligonucleotides without selectivity. Therefore, this product can efficiently degrade all kinds of DNA and RNA (double stranded, single stranded, linear and circular) but without proteolytic activity, which is widely used to remove nucleic acid residues and contamination in biological products.

Universal Nuclease Principle Diagram



Kit Contents

Component	LN201-01 (25 KU)	LN201-02 (50 KU)
Universal Nuclease	100 μl	200 μl

Definition of Enzyme Activity

One unit (U) Universal Nuclease is the amount of enzyme required to change the absorption value of △A260 by 1.0 (equivalent to the complete digestion of 0.7 μg of salmon sperm DNA into oligonucleotides) in a 50 μl reaction system over 30 minutes at 37°C, pH 8.0.

Enzyme Storage Buffer

20 mM Tris-HCl, 2 mM $MgCl_2$, 20 mM NaCl, 50% Glycerol, pH 8.0, @25°C

Recommended Conditions of Use

This product maintains high stability and reactivity under a wide range of conditions (6 M Urea, 0.1 M Guanidine HCl, 0.4% Triton X-100, 0.1% SDS, 1 mM EDTA, 1 mM PMSF) and is compatible with a variety of cell lysates such as RIPA, or protein extraction reagents containing a variety of ionic and nonionic detergents and reducing agents. The following are some of the conditions that this product can tolerate:

Condition Parameter	Optimum Condition	Valid Condition
Mg^{2+}	1~10 mM	1~20 mM
pH	6.0~9.0	4.0~11.0
Temperature	20-40°C	0-50°C
DTT	0~100 mM	>0 mM
β-Mercaptoethanol	0~100 mM	>0 mM
Monovalent Cations (eg. K^+, Na^+)	0~40 mM	0~150 mM
Phosphate Anion ($PO4^{3-}$)	0~10 mM	0~100 mM

*Note: Universal Nuclease activity is ≥90% under the optimum conditions listed in this table, and the enzyme activity is ≥15% under valid conditions.

Recommended Reaction System

Experiment Type	Prepare Electrophoretic Protein Samples	Protein Production	Vaccine Production, Virus Purification	Cell Drug Production
Cell Amount	1×10⁶ cells (10 mg tissue)	1 g wet weight (resuspension 10 ml)	1 L fermentation supernatant	1 L culture
Minimum Enzyme Amount	125 U	250 U	100 U	100 U
Recommended Enzyme Amount	500 U	2,500 U	25,000 U	5,000 U
Reaction Time	Generally, the reaction time is 30~60 minutes at 37°C, or 45 minutes~2 hours at 25°C			

Recommended Usage

Universal Nuclease digests nucleic acids:

1) Add an appropriate amount of $MgCl_2$ to adjust the Mg^{2+} concentration in the range of 1~5 mM, and adjust the pH to 8.0~9.2;
2) Add Universal Nuclease at the ratio of 250 U to digest 1 g of cell (prokaryotic) precipitation and incubate at 37°C for at least 30 minutes. (The operation can also adjust the reaction conditions according to the recommended reaction conditions in the table above.)

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 Universal Nuclease to cell or bacterial lysate to remove nucleic acids from crude extracts, reduce solution viscosity, and increase protein yield;
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. 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;
5. Reduce clumping of human peripheral blood single cells (PBMCs) stored in cell therapy and vaccine studies.

Notes

- If the solution is acidic or alkaline, or contains high concentrations of salts, detergents and 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 enzymatic activity of Universal Nuclease is affected by factors such as ion concentration, reaction temperature and pH. It is recommended to explore the optimum concentration for the first use.

FAQs & Solutions

1. What are the inhibitory conditions for Universal Nuclease?

This product maintains activity under a wide range of conditions, but 1~2 mM Mg^{2+} is critical for its activity. In general, Universal Nuclease activity can be inhibited by high concentrations of salts. For example: the concentration of monovalent cations (such as Na^+, K^+, etc.) > 200 mM will significantly inhibit the enzyme activity, and the concentration of phosphate, ammonium sulfate, guanidine hydrochloride, etc. greater than 100 mM will also reduce the enzyme activity. In addition, if metal chelators such as EDTA are included in the digestion system, the enzyme activity will be inhibited by chelating Mg^{2+} in the system (1 mM EDTA can partially inhibit Universal Nuclease activity, 5 mM EDTA can reduce its activity by approximately 90%). The Universal Nuclease activity can be restored by adding more Mg^{2+} at this point.

2. Is Universal Nuclease compatible with protease inhibitors?

It is compatible. But it should be noted that it is best to choose protease inhibitors that do not contain metal chelators such as EDTA.

3. When the reaction temperature is lower than the optimum temperature of 37°C, how to ensure the digestion effect?

When the reaction system is fixed, the digestion effect of Universal Nuclease mainly depends on the amount of enzyme, reaction temperature and reaction time. When the reaction temperature is low, it is recommended to extend the reaction time appropriately, and try not to add excess Universal Nuclease to improve the digestion effect, because it will cause residual problems caused by Universal Nuclease.

4. How should I control the amount of Universal Nuclease used when processing eukaryotic and prokaryotic cell samples?

The nucleic acid content in eukaryotic cells is much higher than that in prokaryotic cells. So more Universal Nuclease should be added to ensure the degradation effect when processing eukaryotic cell samples. For eukaryotic cell samples, it is recommended to add enzymes at an amount of >2,500 U/g cells, and for prokaryotic cell samples, the amount of enzyme added should not be less than 250 U/g cells. The more the amount of enzyme added, the better the nucleic acid degradation effect, but it is not recommended to improve the digestion effect only by increasing the amount of enzyme. The reason is as above FAQs & Solution 1.

5. What are the storage conditions for this product?

The storage buffer for Universal Nuclease is 20 mM Tris-HCl, 2 mM $MgCl_2$, 20 mM NaCl, 50% Glycerol, pH 8.0, @25°C. This product needs to be stored at -20°C, which can ensure the long-term stability of the enzyme and effectively inhibit the increase of endotoxin content.

Quality Control

Item	Standard
Appearance	Colorless and transparent
Molecular Weight	30 kDa
Isoelectric Point	6.85
Purity	≥99%（SDS-PAGE）
Enzyme Activity	≥250 U/μl
Specific Activity	$\geq 1.0\times10^6$ U/mg protein
Cofactor	1~10 mM Mg^{2+}
Operating pH	Optimum 8.0 (effective operating range 6.0~10.0)
Operating Temperature	Optimum 37°C (effective operating range 0~42°C)
Bacterial Residue	Not detectable
Protease Activity	Not detectable
Endotoxin Content	<0.25 EU/1000 U
Host Protein Residue	≤10 ppm（μg/ml）
Mycoplasma Residue	Not detectable

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Service telephone +86-10-57815020
Service email complaints@transgen.com