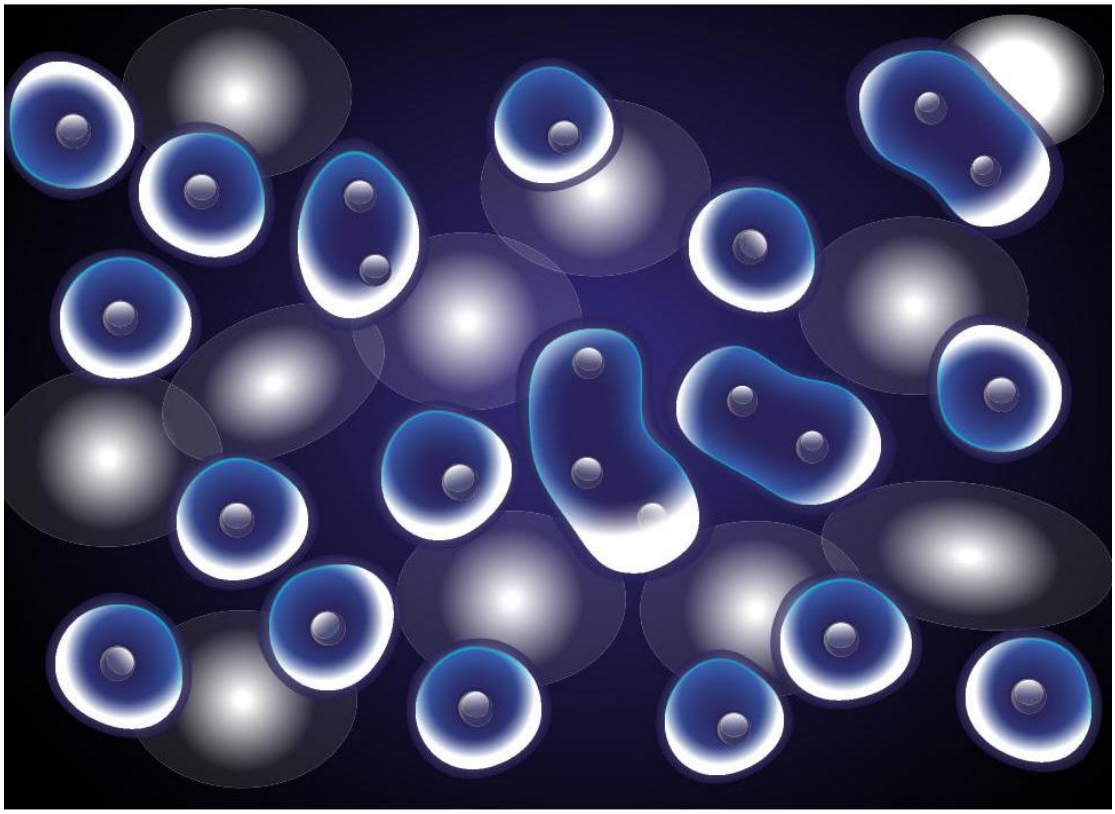


USER Manual of PANNARASE Salt Active Nuclease(SAN)

Shanghai Chaselection Biotechnology Co.,Ltd

www.chaselection.com Marketing@chaselection.com

Introduction of Pannarase Salt Active Nuclease (SAN)

Pannarase SAN is a non-specific endonuclease, which is expressed and purified from the Escherichia coli strain BL 21. This product can degrade any form of DNA and RNA (linear, circular, superhelix) into 3 '- single nucleotide and dinuc leotide, and maintain efficiency under a wide range of operating conditions. It can not only reduce the viscosity of cell sup ernatant and cell lysate in scientific research, but also improve protein purification efficiency and functional research;It ca n also be applied in virus purification, vaccine production, protein and polysaccharide pharmaceutical industries as a host r esidual nucleic acid removal reagent, reducing the level of host residual nucleic acid to a gram (pg), thereby improving the efficacy and safety of biological products; And it can effectively prevent the aggregation of human peripheral blood mon onuclear cells (PBMCs) in cell therapy and vaccine research. This product is provided in the form of sterile liquid enzyme and stored in a buffer solution (25 mM Tris-HCl，5 mM MgCl2，500 mM NaCl，50%Glycerol，pH7.5), and is a colorl ess and transparent liquid.

Name	Cat. No.	Package
Pannarase Salt Active Nuclease	CY006F0010	100,000U/tube
	CYG006F00010	100,000U/tube
Pannarase Salt Active Nuclease GMP Level	CYG006F00050	500,000U/tube
	CYG006F00500	5,000,000U/tube

Unit Definition

One unit is defined as the amount of enzyme that causes a ΔA260 = 1.0 in 30 minutes at 37°C in 25 mM Tris-HCI pH 8.5 (@25° C), 5 mM MgCl2, 500 mM NaCl, and 50 μg/ml calf thymus DNA.

Storage

Stable storage at -20 ℃ to avoid repeated freeze-thaw.

【Note】： Do not place it in an environment of -70 ℃, as freezing can cause loss of enzyme activity.

Properties

Source	*E.coli*
Molecular weight	16.8 kDa
Purity	＞95% (SEC-HPLC)
Specific activity	1.98×10^{6} U/mg
Endotoxin	< 1 EU/mg
Protease	Undetected

Working Range

Condition	optimum range*	valid range#
Mg^{2+}	0.55-5 mM	0-10 mM
Ca^{2+}	0.5-10 mM	0-10 mM
pH	8.5-10	7.0-11.0
Na^{+}	0-150 mM	0-500 mM
Temp	35℃	0-42℃

*“ optimum range” defined as above 90% of activity

**“ valid range” defined as above 15% of activity

Instructions for use

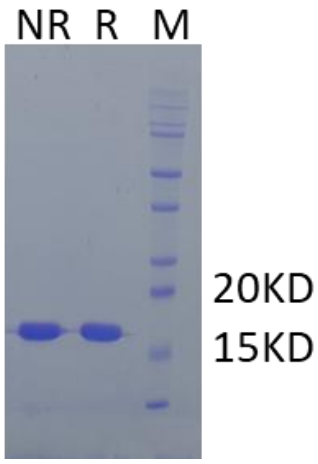
1.Escherichia coli crushing solution：In order to achieve the goal of reducing viscosity, the amount of nuclease added must be determined based on the bacterial concentration of the broken liquid. It is recommended to add a nuclease amount of 1:1000 to 5:1000 if the bacterial concentration is 50%, which is 500000 to 250000 U/L. If the bacterial concentration is 5%, it can be added in a ratio of 1:10000 to 5:10000.

2. For cell lysate, 500 units of nuclease can be added to 10^6 to 10^7 cells.

3. For purified adenovirus or viral vaccines, due to their relatively low DNA content, they can be added at a final concentration of 5 units per milliliter, ranging from 1/10000 to 5/10000.

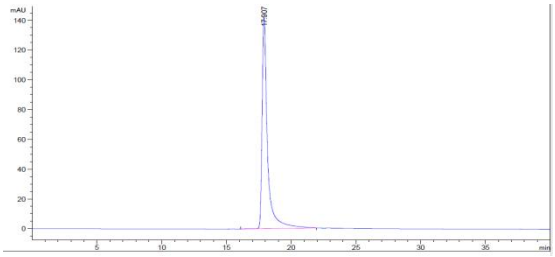
Product characterization of Pannarase SAN

SDS-PAGE :



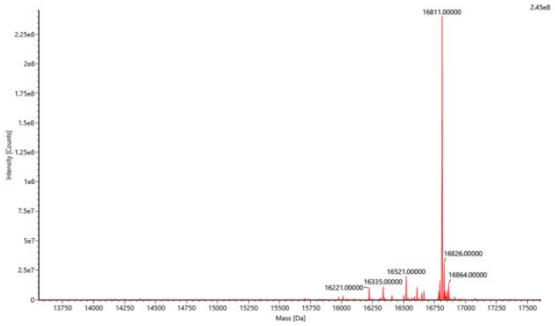
Non reducing and reducing SDS-PAGE showed a protein molecular weight of approximately 16kDa, and no foreign proteins were found.

SEC-HPLC :



Size exclusion chromatography shows high purity and no aggregation generation

LC-MS:

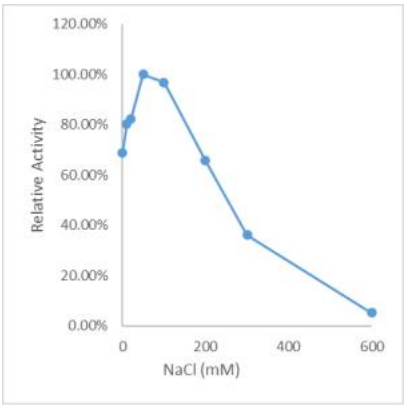


Complete protein analysis by liquid chromatography mass spectrometry, confirming that the precise molecular weight of the protein is consistent with the theoretical value

Performance of Pannarase SAN

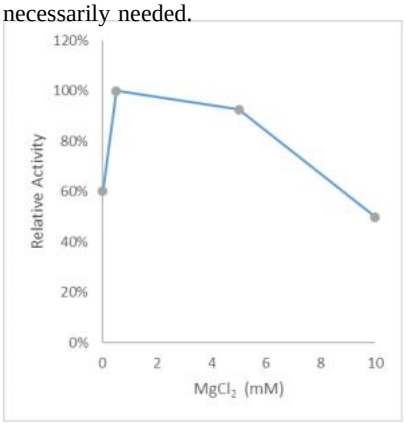
1. The effect of Na^+ ions on the activity of Pannarase enzyme

Pannarase enzymea has high activity under moderate Na^+ concentration conditions, with 36.2% activity still at 300 mM, but the enzyme activity is basically lost when the concentration exceeds 600 mM.



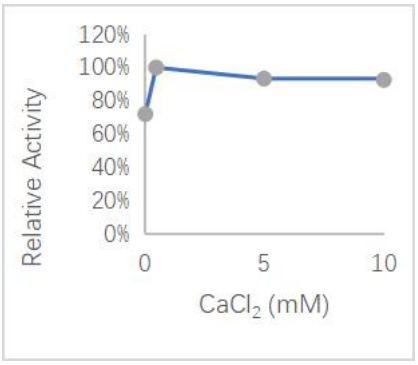
2. The effect of Mg^{2+} ions on the activity of Pannarase enzyme

A concentration of 1mM Mg^{2+} can raise the activity of Pannarase endonuclease by about 40%. But Mg^{2+} is not necessarily needed.



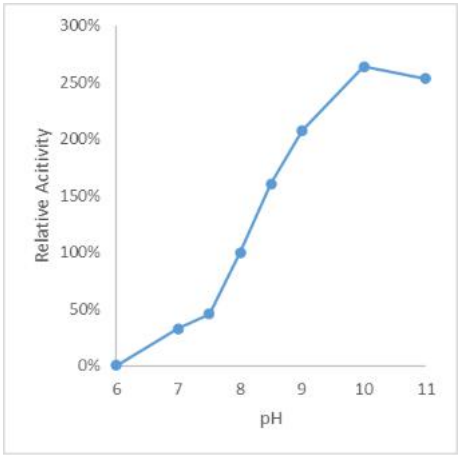
3. The effect of Ca^{2+} ions on the activity of Pannarase enzyme

A concentration of 1mM Ca^{2+} can raise the activity of Pannarase endonuclease by about 28%. But Ca^{2+} is not necessarily needed.



4. Comparison of Pannarase enzyme activity under different pH conditions

The optimal enzyme activty of Pannarase endonucleas is between pH 8.0 to 11.0.



5. Genomic DNA enzyme digestion experiment

The substrate DNA (genomic DNA of E. coli strain BL21(DE3)) was diluted with assay buffer(50 mM Tris-HCl pH 8.0, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1mg/mL BSA) into 1 mg/mL. Incubatethesubstrate with different amount of Pannarase at 37℃ for 30 min. The DNA fragment wasanalyzed by agarose gel electrophoresis and photographed.