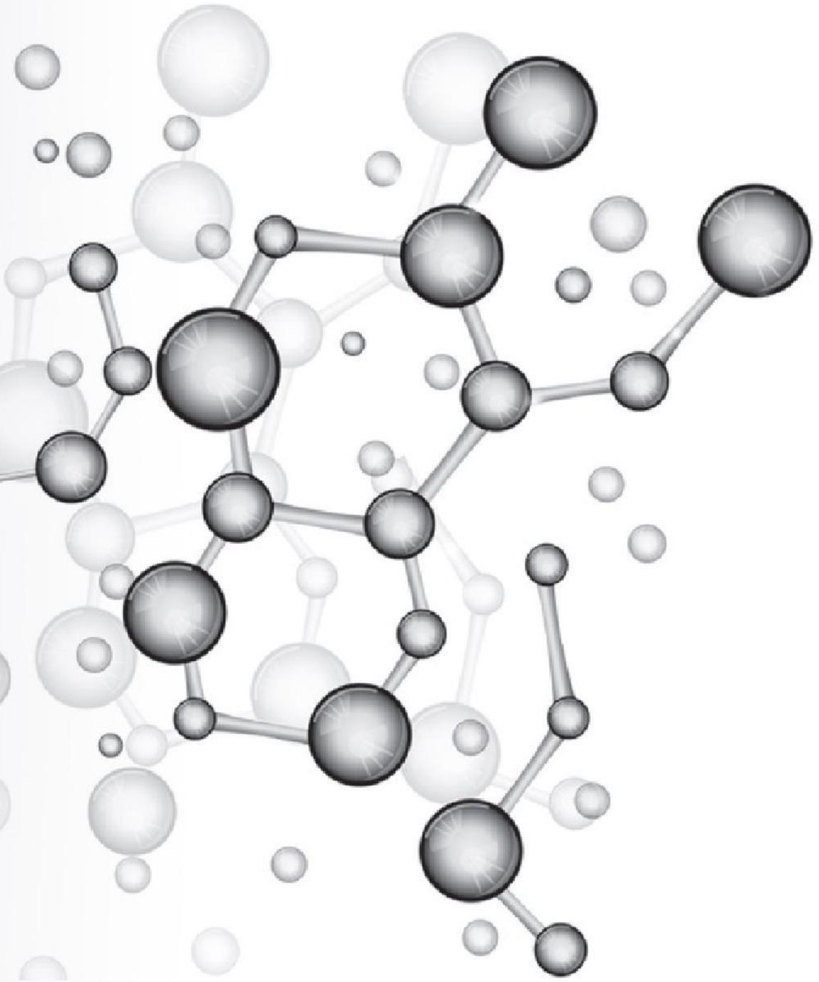
CHASELECTION

Pannarase

User Manual of Pannarase Universal Nuclease



CHASELECTION

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Introduction of Pannarase Universal Nuclease

Pannarase (EC 3.1.30.2) is a non-specific endonuclease derived from Serratia marcescens. The enzyme is expressed and purified from Escherichia coli strain W3110, which is a mutant strain of K12 strain and contains pET41 production plasmid. Structurally, the protease is a homodimer with a single subunit molecular weight of 26.8 kDa, consisting of 246 amino acids and two pairs of active disulfide bonds. Its activator is Mg^{2+}。 This enzyme can degrade any form of DNA and RNA (linear, circular, superhelix) into 3-5 bp oligonucleotides and maintain efficiency under a wide range of operating conditions.

We provide Panarase Universal Nucleases with purity>99% (SEC-HPLC) and specific activity up to 2 $\times 10^6$ U/mg, endotoxin<1 EU/mg, and no detectable protease activity or viral pollutants.

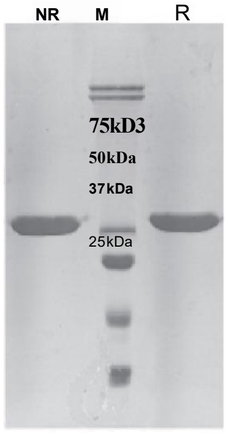
Cat. No.	Name	Package
CYG002F0010	Pannarase Universal Nucleaese，GMP Level	100,000U/tube
CYG002F0050	Pannarase Universal Nucleaese，GMP Level	500,000U/tube
CYG002F0500	Pannarase Universal Nucleaese，GMP Level	5,000,000U/tube

Unit Definition: By measuring its ability to cleave herring sperm DNA substrate (Herring Sperm DNA, Sigma, catalog number D7290). one unit is defined as a decrease of 1.0 in A260nm (equivalent to complete digestion of 37ugDNA) within 30 minutes under reaction conditions of 37 ℃, pH 8.0, with a specific activity>1.0 × 10^6U/mg.

Storage: 20mM Tris HCl, pH 8.0, 20mM NaCl, 2mM $MgCl_2$, 50% Glycerol, stable storage at -20 ℃, avoiding repeated freeze-thaw.

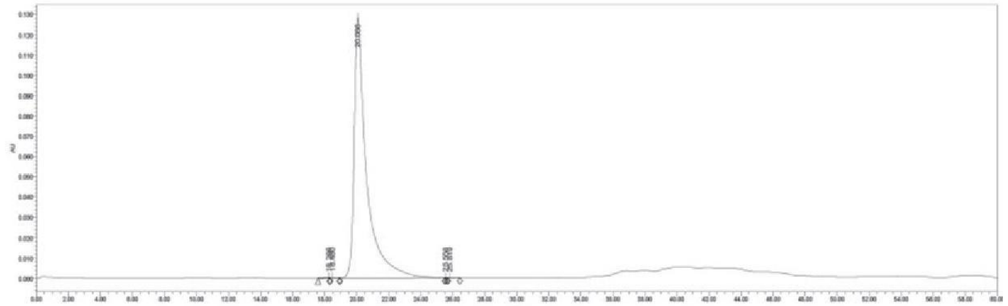
Characterization of Pannarase Universal Nuclease

SDS-PAGE:



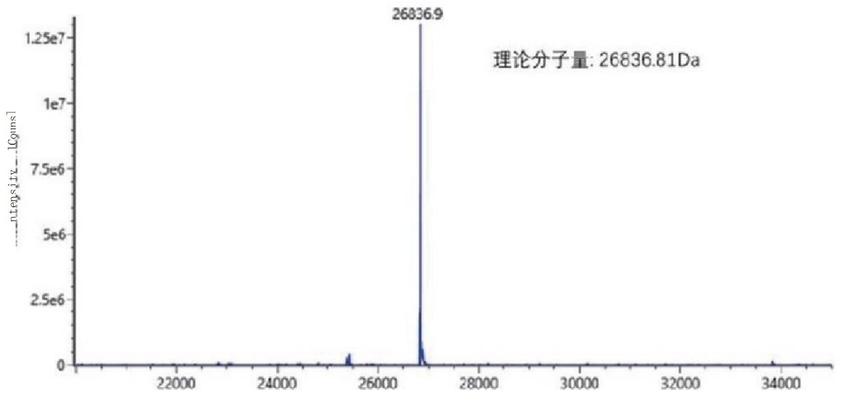
Non reducing and reducing SDS-PAGE showed a protein molecular weight of approximately 27 kDa, and no foreign proteins were found.

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

Application of Pannarase Universal Nuclease

1.Removal of DNA contamination in vaccine and virus sample preparation;

2.Removing nucleic acid contamination during protein extraction: Panarase nuclease can effectively reduce sample viscosity during recombinant protein purification or tissue cell sample protein extraction, which is beneficial for downstream operations;

3.The combination of cell or bacterial lysis solution can remove nucleic acid from the crude extract, reduce solution viscosity, and increase protein production;

4.Reduce the agglomeration phenomenon of stored PBMCs;
5.Degradation of nucleic acids and beneficial for preparation of high-quality inclusion systems for insoluble proteins before refolding
6. Effectively removing the impact of negatively charged nucleic acids on bidirectional SDS-PAGE protein samples, improving protein separation efficiency, and enhancing 2-DE resolution

Note

1.Pannarase univeral nuclease can maintain its activity under the following reaction conditions:

condition	optimum range*	valid range**
Mg^{2+}	1-2mM	1-10mM
pH	8.0-9.2	6.0-10.0
Na+/K+ concentration	0-20 mM	0-150 mM
Temperature	37℃	4-42℃
(Dithiothreitol)DTT	0-100 mM	0-300 mM
2-mercaptoethanol	0-100 mM	0-300 mM
PO^{3}	0-10 mM	0-100 mM

* “optimum range” defined as above 90% of activity
**“valid range” defined as above 15% of activity

Note: Specific parameters can be found in the Panarase product performance section
2. The activity is optimal at low salt levels (0-20mM NaCl). If it is used in a high salt environment , the amount can be
appropriately increased or the incubation time can be extended. Exceeding 300mM can lead to the loss of actitity.
3. Pannarase nuclease can be compatible with protease inhibitors, but protease inhibitors containing EDTA formulas should be used with caution. When EDTA concentration is greater than 1mM, it will inhibit the activity of nuclease;
4. In the presence of denaturants (such as 4M urea), the enzyme remains active and can be directly added to the protein lysis solution;
5. The optimal temperature for Panarase nuclease is 37 ℃. If low-temperature operation is required, the incubation time can be appropriately extended to compensate without increasing the enzyme amount (in the case of a fixed system, the digestion effect of Pannarase nuclease mainly depends on the enzyme dosage, reaction temperature, and reaction time. When the reaction temperature is low, to avoid residual problems,it is more recommended to extend the reaction time to ensure digestion effect);
6. If it is necessary to inhibit the activity of Panarase nuclease, it can be achieved by adding inhibitors such as high salt and EDTA. The inhibitory effect of EDTA is reversible and can be relieved by adding a large amount of $MgCl_2$.

Instructions for the use of Panarase universal nuclease

1. Escherichia coli breaking solution: In order to reduce viscosity, the amount of nuclease added must be determined based on the bacterial concentration of the breaking solution. If the bacterial concentration is 50%, it is recommended to add a nuclease amount of 1:1000-5:1000, which is 500k-2500k U/L. If the bacterial concentration is 5%, it can be added in a ratio of 1:10000 to 5:10000.
2. For eukaryotic cell lysate, 500 units of nuclease can be added to 10-107 cells, and the initial addition amount can be added in a ratio of 1:1000.
3. 3. For purified adenovirus or viral vaccines, due to their relatively low DNA content, they can be added at a ratio of 1: 10000 to 5:10000.

Note:

1, The optimal operating conditions for the enzyme are pH 8.0-9.2, 37 ℃, and 30 minutes;

2, Magnesium ions are necessary for enzyme activity, and a concentration of 2 mM is recommended. If there are metal chelating agents such as EDTA in the sample, they need to be removed or neutralized by addding excessive magnesium ions.

3. The activity of the enzyme is relatively stable, and it will not have a significant impact in the short term when stored at room temperature. For long-term storage, it needs to be stored in a -20 ℃ refrigerator. The enzyme solution will not freeze as the protective solution contains 50% glycerol. Dilute the enzyme with glycerol free buffer and use it as soon as possible. It is not recommended to use it after freezing;

4. For the purpose of reducing viscosity only, nuclease can be directly added and stirred slowly at room temperature for 30 minutes.

Q&A:

Q1： If I have used Panarase nuclease and forgot to store it in a -20 ℃ refrigerator, can the nuclease still be used after being stored at room temperature for the weekend?

A: No problem, Panarase nuclease is very stable, and even after being stored at 25 ℃ for several months, it still has enzyme activity over 90%.

Q2: Protein is insoluble and requires purification under denaturation conditions. Can Pannarase nuclease digest nucleic acids in a urea environment?

A: The activity of nuclease increases at a urea concentration of 6M firstly, and then decreases with time; When urea is 7M, Pannarase nuclease undergoes denaturation and inactivation after 15 minutes. However, most nucleic acids have already been degraded before enzyme inactivation. The effect of 7M urea can be compensated by increasing the concentration of nuclease usage.

Q3： How to ensure the digestion effect of Panarase nuclease when the reaction temperature is below 37 ℃?

A ： In the case of a fixed system, the digestion effect of Panarase nuclease mainly depends on the enzyme dosage, reaction temperature, and reaction time. When the reaction temperature is low, it is more recommended to extend the reaction time to avoid residual problems caused by excessive Panarase nuclease.

Q4. How to inactivate Pannarase nuclease? How to remove it?

A: A: The use of EDTA chelating metal ions can reversibly inhibit nuclease activity, and extreme conditions can cause irreversible inactivation, such as100mM NaOH, treated at 70 ℃ for 30 minutes, etc. Nuclease can be separated from the target product using an anion exchange column.

Q5. How should the product be diluted?

A: A: The normal recommended dilution ratio (final concentration) is 1:1000-1:20000. The original storage solution contains 50% glycerol, and the enzyme solution will not freeze at -20 ℃. However, the enzyme diluted with a buffer solution without glycerol needs to be used as soon as possible. It is not recommended to use it again after freezing.

Q6: Can the product reduce usage?

A: If you want to reduce the dosage, you can increase the reaction temperature or extend the time as appropriate

Q7： How much is used each time after enzyme dilution, how to test the gradient, and how much is added initially?

A: It depends on different experimental requirements. The dosage can be between 1:100 and 1:10000. The demand for eukaryotic cells is higher than that of prokaryotic cells because the nucleic acid content in eukaryotic cells is high. The initial addition amount of eukaryotic cells can be added in a ratio of 1:1000, while prokaryotic cells can be added in a ratio of 1:10000; The amount of DNA added during digestion at 4 ℃ is higher than that during room temperature digestion, as the Panarase activity is higher at room temperature. In ordinary Co IP experiments, protein expression experiments can be slightly increased, while experiments with high requirements for DNA residue can be added in a ratio of 1:100.

Q8: What are the reaction buffers?

A: Commonly used biological buffers, such as TBS, MOPS (pH6-8), etc.

Removal of Pannarase Universal Nuclease

Pannarase Nuclease is also a protein, and if added to bacteria or cell lysates, it will be removed like other miscellaneous proteins with the purification step. If it is added to a virus or viral vaccine, because of the huge difference in molecular weight between nuclease and virus, it can be removed by gel filtration (such as Sepharose6FF), and the residual amount of nuclease can be detected by a kit.

In downstream purification operations, Pannarase universal nucleases can be removed from the production process through various methods, such as tangential flow filtration TFF or chromatography. The successful removal of Pannarase universal nuclease was demonstrated by ELISA detection of total residual endonucleases (active and inactive). Pannarase is easy to remove from the filtrate, and can be removed from the process by using TFF and selecting appropriate membrane interceptors to a concentration below the detectable limit.
According to the purification process, ion exchange chromatography can be used instead of TFF or in conjunction with TFF to remove Pannarase universal nuclease. As is well known, ion chromatography can remove residual endonucleases from pharmaceutical products. Due to the Pannarase nuclease PL being 6.85, when samples containing a small amount of Pannarase enzyme and binding buffer contain appropriate concentrations of NaCl, the Pannarase enzyme usually flows through the anion exchange chromatography (AEX) column in a flow through mode, and the target protein binds to the anion chromatography (AEX) column, so they are eluted separately. Table 1 lists several anion exchange resins suitable for various samples and equilibrium buffer solutions, which can be used for the removal of Pannarase omnipotent nucleases.

Cation exchange chromatography (CEX) is also effective in removing Pannarase universal nucleases, but the operating range may be small. Table 2 provides a reference list of cation exchange chromatography media and conditions suitable for the removal of Pannarase universal nuclease.

Table 1: Anion exchange chromatography packing for removing Panarase universal nuclease

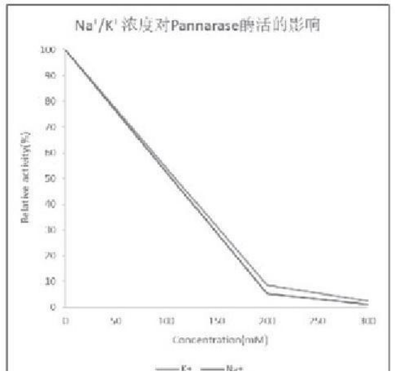
Anion-exchange chromatography	pH	Sample and equilibrium buffer	Pannarase Universal nuclease	BSA
QSepharose	7.0	50 mM Tris/200 mM NaCl	uncombined	uncombined
Q Sepharose	7.0	50 mM Tris /50 mM NaCl	uncombined	combined
QSepharose	8.0	50mM Tris/250mM NaCl	uncombined	uncombined
QSepharose	8.0	50 mM Tris/100mM NaCl	uncombined	combined
QSepharose	9.0	50mM Tris/200 mM NaCl	uncombined	Partial combination
Q Sepharose	9.0	50mM Tris/100 mM NaCl	uncombined	combined
DEAE Sepharose	7.0	50 mM Tris /200 mM NaCl	uncombined	uncombined
DEAE Sepharose	7.0	50 mM Tris /50 mM NaCl	uncombined	combined
DEAE Sepharose	8.0	50mM Tris/250 mM NaCl	uncombined	uncombined
DEAE Sepharose	8.0	50 mM Tris/100 mM NaCl	uncombined	combined
DEAE Sepharose	9.0	50 mM Tris/250mM NaCl	uncombined	uncombined
DEAE Sepharose	9.0	50 mM Tris /50 mM NaCl	uncombined	combined

Table 2: Cation exchange chromatography packing for removal of Pannarase universal nuclease

Cation-exchange chromatography	pH	Sample and equilibrium buffer	Pannarase Universal Nuclease
SP Sepharose	6.0	20 mM Phosphate /100 mM NaCl	combined
SP Sepharose	6.0	20 mM Phosphate /200 mM NaCl	uncombined
SP Sepharose	5.0	20 mM NaAc /200 mM NaCl	combined
SP Sepharose	5.0	20 mM NaAc/700mM NaCl	uncombined
SP Sepharose	4.0	20 mM NaAc /300 mM NaCl	combined
SP Sepharose	4.0	20 mM NaAc/700 mM NaCl	uncombined
CM Sepharose	6.0	20 mMPhosphate	uncombined
CM Sepharose	5.0	20mM NaAc 40mM NaCl	combined
CM Sepharose	5.0	20mM NaAc100mM NaCl	uncombined
CM Sepharose	4.0	20mM NaAc150mM NaCl	Partial combination
DEAE Sepharose	4.0	20mM NaAc 400mM	uncombined

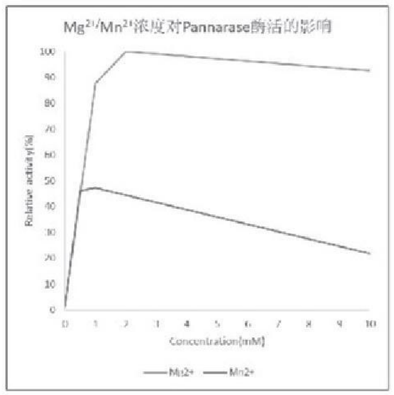
Performance of Pannarase

1. The Effect of Monovalent Cations on Pannarase Activity



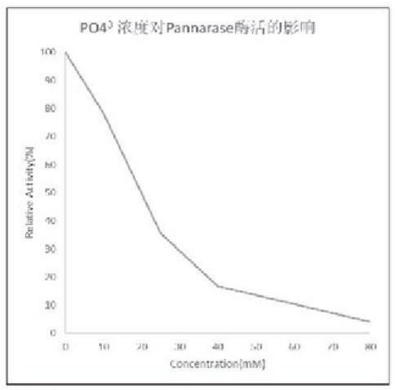
Nat/K+strongly inhibits the endonuclease activity of Panarase, and the enzyme activity is completely lost when the concentration exceeds 300mM.

2.The effect of divalent cations on the activity of Pannarase enzyme



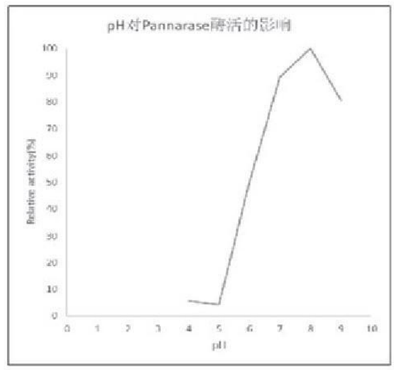
1-2mM Mg^{2+}/Mn^{2+} is necessary for Pannarase endonuclease activity, Mg^{2+} is the preferred choice as it can achieve optimal enzyme activity.

3. The effect of PO43- concentration on Pannarase enzyme activity



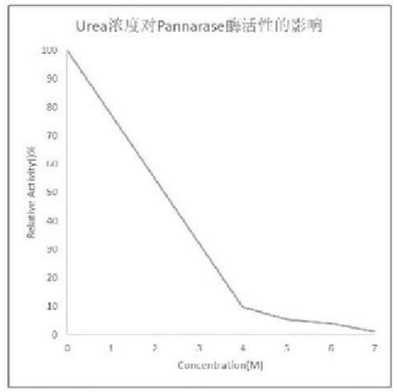
PO43- strongly inhibits the activity of Panarase enzyme, and around 80mM can completely inactivate Panarase enzyme. (The incubation buffer for this experiment is Tris PO43 buffer)

4. Comparison of Panarase enzyme activity under different pH conditions



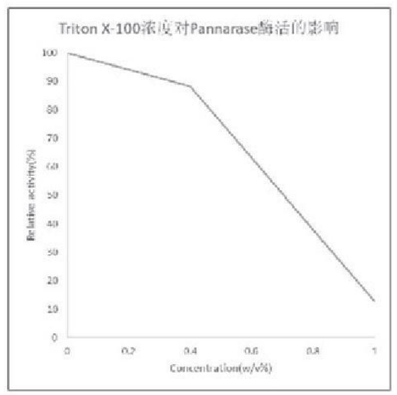
The optimal pH for Pannarase enzyme activity is 8.0-9.2.

5. The effect of Urea concentration on Panarase enzyme activity



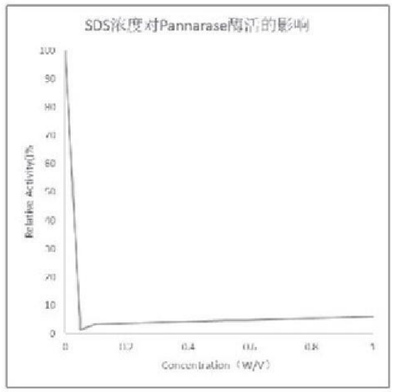
Under low concentration Urea conditions, Pannarase enzyme can still maintain high activity, and at high concentration Urea, Pannarase enzyme can also be used to remove nucleic acids. At this time, the effect of low enzyme activity can be compensated by increasing the enzyme amount.

6. The effect of Triton X-100 concentration on Pannarase enzyme activity



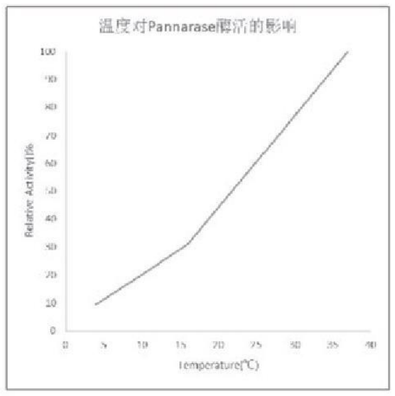
Add corresponding detergents to the standard enzyme activity measurement reaction system to evaluate the effect of detergents on Panarase enzyme activity. When the concentration of TritonX-100 is less than 0.4%, it has no effect on Panarase enzyme activity

7. The effect of SDS concentration on Pannarase enzyme activity



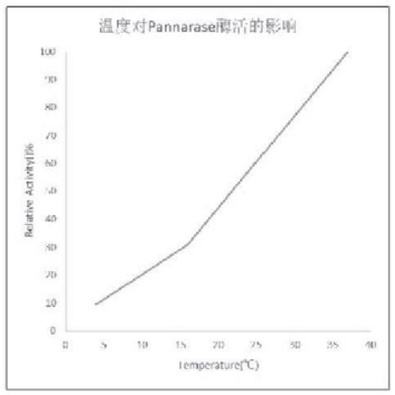
SDS has a strong inhibitory effect on Pannarase nuclease activity, even at a concentration of 0.05%, it can lead to complete inactivation of enzyme activity.SDS

8. The effect of temperature on Pannarase enzyme activity



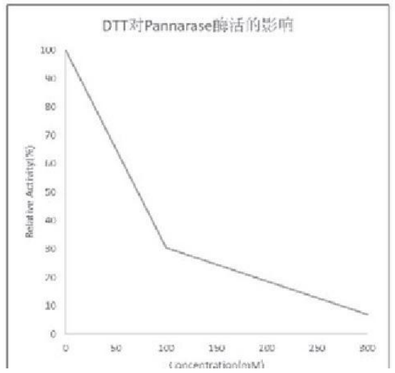
37 ℃ is the optimal temperature for Panarase enzyme, and the enzyme activity will decrease with the decrease of temperature. If the enzyme needs to be used at low temperature, the incubation time can be appropriately extended. It is not recommended to increase the amount of enzyme involved in enzyme removal treatment.

9. Different concentrations of β- Mercaptoethanol E on Pannarase enzyme activity



Pannarase enzyme can tolerate higher concentrations of β- Mercaptoethanol, Panarase enzyme can maintain over 90% activity under 0-100mM βME,, while β- ME concentration is greater than 100mM, the Panarase enzyme remains active.

10. The effect of different concentrations of DTT on the activity of Panarase enzyme



Pannarase enzyme can tolerate higher concentrations of DTT. In the presence of 0-100mM DTT, the Panarase enzyme still maintains over 90% activity, while when the DTT concentration is below 300mM, the Panarase enzyme still remains active.

11. Plasmid DNA cleavage assay

Plasmid DNA cleavage assay

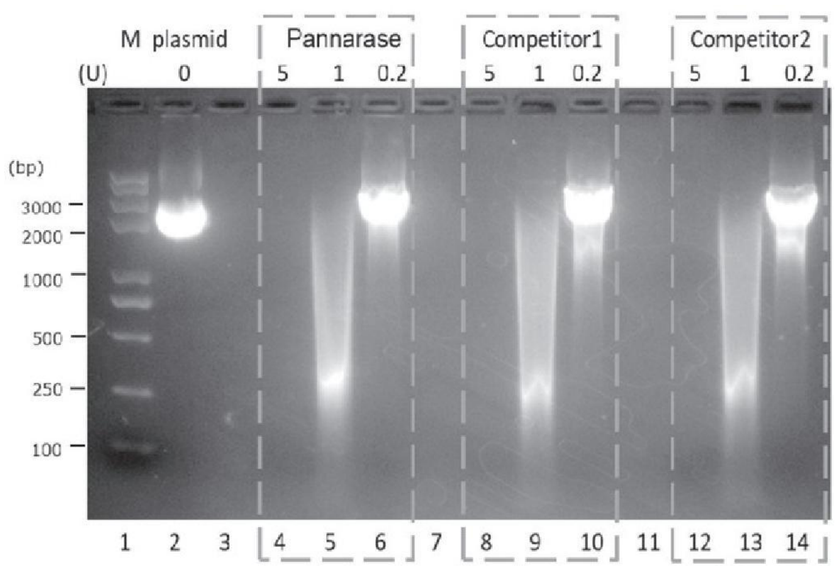


Fig. Comparison of the Pannarase and other nucleases in different amount of nuclease by plasmid DNA cleavage assay.

Dilute the base material particles with measuring buffer (50 mM Tris HClpH8.0,1mM $MgCl_2$, 0.1mg/mLBSA) to 1mg/mL. Incubate the substrate with different units of Pannarase and other nucleases at 37 ℃ for 30 minutes. DNA fragments were analyzed by agarose gel electrophoresis and photographed.