Tinzyme Co., Limited

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MnlI

Product Number: RS02

Shipping and Storage

Store at -20ºC, valid for two years.

Components

Component	RS02
MnlI	50μl
10×Cut Buffer	1ml
Load 10×Cut Buffer	1ml

Description

MnlI is a high-quality restriction endonuclease that has undergone genetic engineering recombination and can quickly complete DNA cleavage using only one buffer in 5-15 minutes.It is suitable for rapid endonuclease digestion of plasmid DNA, PCR products, or genomic DNA.

Enzyme activity detection: Under the optimal reaction temperature, 1μl MnlI can completely digest 1μg of DNA in a 20μl reaction system within 15 minutes.

Long term enzyme digestion detection: At the optimal reaction temperature, 1μl MnlI was incubated with 1μg of DNA for 3 hours, and no other nuclease contamination or non-specific substrate degradation caused by star activity was detected. Delayed enzyme digestion may result in star activity.

Digestion-Connect-Reenzyme digestion detection: At the optimal reaction temperature, use 1μl MnlI to digest the substrate and recover the enzyme digestion product. Use an appropriate amount of T4 DNA Ligase at 22℃ to reconnect the enzyme digestion product. After the connection product is recovered again, use the same endonuclease to re cut the connection product.

The basic information of MnlI is as follows:

Recognition sequence	Isoschizomer	Enzyme digestion temperature	Deactivation conditions	Methylation interference?
5'-CCTCN7^-3' 3'-GGAGN6^-5'	NO	37℃	80℃ 20min	Sometimes

The activity of MnlI in different reaction buffer solutions (buffer compatibility) is as follows:

10×Cut Buffer	Load 10×Cut Buffer	Thermo FastDigest Buffer	NEB CutSmart® Buffer	Takara QuickCut™ Buffer
100%	100%	100%	100%	100%

Please refer to the table below for the methylation effects of MnlI recognition sites:

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	No effect	No effect	Sequences may overlap and Shear blocking

Features

1. Enzymatic digestion can be completed within 5-15 minutes;
2. The entire series of endonucleases share a common enzyme cutting buffer, greatly simplifying the enzyme cutting reaction system and facilitating double or multiple enzyme cutting;
3. Many modified enzymes, such as Alkaline Phosphatase、Antarctic Phosphatase、T4 DNA Ligase、T4 Polynucleotide Kinase、T4 PNK (3' phosphatase minus), are 100% compatible with the buffer, allowing for compatibility between reaction systems such as "enzyme cleavage linkage" and "enzyme cleavage modification linkage", supporting one tube reactions;
4. Good enzyme activity redundancy makes it easy to cope with substrate excess or difficult template enzyme cleavage.

Note

For Research Use Only

1. Endonucleases should be stored in an ice box or ice bath when used, and should be immediately stored at -20ºC after use.
2. If it is found that the expected cleavage site cannot be cleaved, please confirm whether there is a methylation interference issue.
3. Homolytic enzymes may have different sensitivities to different methylation modifications, and when encountering potential methylation interference issues, homolytic enzymes can be attempted.
4. This product is only for scientific research purposes by professionals and cannot be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
5. For your safety and health, please wear laboratory clothes and disposable gloves when operating.

Protocol

1. When performing single enzyme digestion, the following reaction system can be referred to for operation on an ice bath.

Reagent	Plasmid DNA	PCR Product	Genomic DNA
Ultrapure Water	(17-x)μl	(26-x)μl	(40-x)μl
10×Cut Buffer or Load 10×Cut Buffer	2μl	3μl	5μl
Substrate DNA	xμl(up to 1μg)	xμl(~0.2μg)	xμl(5μg)
MnlI	1μl	1μl	5μl
Total volume	20μl	30μl	50μl
Incubate at 37℃	15min	15-30min	30-60min

Note:The above reaction system is suitable for enzymatic digestion of purified PCR products. The unpurified PCR product has a certain ion strength and pH, and the addition of 10×Cut Buffer can be appropriately reduced to 2μl. However, due to the fact that many DNA polymerase enzymes also have exonuclease activity, it can affect the cleavage products. Therefore, the following steps require ligation, cloning, and other operations. It is recommended to purify the PCR products before enzymatic cleavage.

1.1. After adding various liquids in sequence according to the above table, use a pipette to gently suck or flick the tube wall to mix well (do not vortex mix), and then momentarily centrifuge to settle the liquid to the bottom of the tube.

1.2. Incubate at 37℃ for 15 minutes (plasmid), 15-30 minutes (PCR product), or 30-60 minutes (genomic DNA). Water bath is preferred for enzyme digestion reactions, and the reaction temperature is usually more constant.

1.3. Incubation at 80℃ for 20 minutes can inactivate the enzyme and stop the reaction (optional).

2. When using double or multiple enzyme digestion, the reaction system can be set according to the following principles based on the enzyme digestion reaction system settings in the reference form.

2.1. The dosage of each fast endonuclease is 1μl, and the reaction system should be appropriately expanded as needed;

2.2. The total volume of all rapid endonucleases shall not exceed 1/10 of the total reaction system;

2.3. If the optimal reaction temperature for several fast endonucleases used is different, the enzyme with the lowest optimal temperature should be used for enzymatic digestion first, and then the enzyme with the highest optimal temperature should be added for enzymatic digestion reaction at its optimal reaction temperature.