Tinzyme Co., Limited

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XmaI

Product Number: REXM01

Shipping and Storage

Stored at -20℃, valid for two years.

Components

Component	Specifications
XmaI	100μL
10×FlashCut™ Buffer	1mL
Easy-Load™ 10×FlashCut™ Buffer	1mL

Description

Our company's XmaI rapid endonuclease is a series of high-quality restriction endonucleases that have been genetically engineered and can quickly complete DNA cleavage using only one buffer within 5-15 minutes.

Application

Suitable for rapid enzymatic digestion of plasmid DNA, PCR products, or genomic DNA.

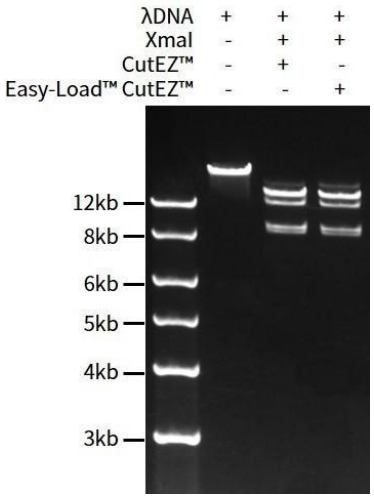
Features

1. Enzyme digestion can be completed within 5-15 minutes
2. This series of endonucleases share a common enzyme cleavage buffer FlashCut™ Buffer, Greatly simplifying the enzyme digestion reaction system, facilitating dual enzyme digestion or multi enzyme digestion;
3. Targeting different enzymes in FlashCut™ The problem of differences in activity in Buffer has been addressed by adjusting the concentrations of different enzymes. Therefore, the enzyme digestion reaction can be carried out uniformly at a dosage of 1μL enzyme per 20μL system;
4. Alkaline Phosphatase, Antarctic Phosphatase, T4 DNA Ligase, T4 Polynucleotide Kinase, T4 PNK (3' phosphatase minus) Many modifying enzymes are 100% compatible with FlashCut™ Buffer enables compatibility between reaction systems such as' enzyme cleavage linking 'and' enzyme cleavage modification linking ', supporting one tube reactions;
5. Good enzyme activity redundancy makes it easy to cope with substrate excess or difficult template enzyme digestion.

Enzyme activity detection

At the optimal reaction temperature, in a 20μL reaction system, 1μL XmaI can completely digest 5μg of λ DNA containing three XmaI cleavage sites within 15 minutes.

The 20μL reaction system contains 5μg of λ DNA (48.5kb), and as shown in the figure, 1μL XmaI is added or not added, respectively, using 1× FlashCut™ Buffer and 1×Easy-Load™ 10× FlashCut™ Buffer, incubate at 37℃ for 15 minutes for enzyme digestion reaction, then incubate at 65℃ for 20 minutes to inactivate the enzyme, followed by electrophoresis and nucleic acid staining using NA Red (D0128/D0130), followed by fluorescence imaging analysis. The DNA marker used is DNA Ladder (0.2-12kb, 12 bands) (D0110). The actual detection effect may vary due to differences in experimental conditions, detection instruments, etc. The effect shown in the figure is for reference only.



Quality control

1. Long term enzyme digestion detection: Incubate 1μL XmaI with 1μg λ DNA for 3 hours at the optimal reaction temperature, and no non-specific degradation of the substrate caused by other nucleases contamination or star activity was detected. Delayed enzyme digestion may result in star activity.
2. Enzyme digestion ligation re digestion detection: At the optimal reaction temperature, use 1μL XmaI 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 recovering the ligation product again, use the same endonuclease to cleave the ligation product again.
3. Detection of non-specific endonuclease activity: at the optimal reaction temperature, 1μL XmaI and 1μg super spiral plasmid DNA were incubated together for 4h, and agarose gel electrophoresis was used to detect that the plasmid DNA was still in the super spiral state.
4. Blue and white spot detection: The vector containing a single lacZ α gene was digested with 1μL XmaI, reconnected, and transformed into competent E. coli cells, and coated on LB medium plates containing corresponding antibiotics, IPTG, and X-gal. Products with correct connections will grow blue colonies, while products with incorrect connections (i.e. incomplete DNA end incisions) will grow white colonies. For this series of restriction enzymes, the proportion of white colonies should be less than 1%.

Protocol

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

Reagent	plasmid DNA	PCR products	Genomic DNA
ddH_2O	(17-x)μL	(26-x)μL	(40-x)μL
10×FlashCut™ Buffer orEasy-Load™ 10×FlashCut™ Buffer	2μL	3μL	5μL
Substrate DNA	xμL(upto1μg)	xμL(~0.2μg)	xμL(5μg)
XmaI	1μL	1μL	5μL
Total	20μL	30μL	50μL
Incubateat37℃	15min	15-30min	30-60min

Note: The above reaction system is applicable for enzyme digestion of purified PCR products. The unpurified PCR product has a certain ionic strength and pH, the amount of 10×FlashCut™ Buffer added can be appropriately reduced to 2μL. However, due to the simultaneous exonuclease activity of many DNA polymerases, 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 cleavage. According to the above system for enzyme digestion of plasmids, there may be a small amount of incomplete digestion of plasmids in the cut state. To achieve complete enzymatic cleavage of substrates using XmaI, the DNA concentration in the reaction system must not be less than 50μg/mL.

1.1. After adding various liquids in sequence according to the table above, use a pipette to gently suck or tap the tube wall to

mix (do not vortex), and then centrifuge instantly 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). It is recommended to use a water bath as the preferred method for enzyme digestion reactions, as the reaction temperature is usually more constant.

1.3. Incubate at 65℃ for 20 minutes to inactivate the enzyme and stop the reaction (optional).

2. When performing 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 rapid endonuclease is 1μL, and the reaction system should be appropriately expanded as needed;

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

2.3. If the optimal reaction temperatures for the several rapid endonucleases used are different, the enzyme with the lower optimal temperature should be used first for digestion, and then the enzyme with the higher optimal temperature should be added for digestion reaction at its optimal reaction temperature.

Basic information of XmaI rapid endonuclease

Identify sequence	Isoschizomer	Enzyme digestion temperature	Inactivation conditions	Methylation interference?
5'-C^CCGGG-3' 3'-GGGCC^C-5'	Cfr9I, TspMI, SmaI	37℃	65℃ 20min	Sometimes there is interference

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	Shear obstruction	no effect	no effect

Activity in different reaction buffers

FlashCut™ Buffer	Easy-Load™ CutEZ™ Buffer	Thermo FastDigest Buffer	NEB CutSmart® Buffer	Takara QuickCut™ Buffer
100%	100%	100%	100%	100%

Note: The activity data comes from the detection under the restriction enzyme standard reaction system.

Note

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