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FlashCut™ KpnI

Product Number: RE0541

Shipping and Storage

-20℃.

Components

Component	Specifications
FlashCut™ KpnI	200μL
10× FlashCut™ Buffer	2×1mL
10× FlashCut™ Color Buffer	2×1mL

Description

FlashCut™ Rapid endonucleases are a series of genetically engineered restriction endonucleases that are suitable for rapid enzymatic cleavage of plasmid DNA, PCR products, or genomic DNA. All FlashCut™ Rapid endonucleases have excellent activity in both FlashCut™ and FlashCut™ Color Buffer, and can complete enzyme cleavage within 5-15 minutes. In addition, Baishimei dephosphorylation and ligation reagents are available on FlashCut™ Buffer has 100% activity and supports one tube reaction, enhancing the experience of "enzyme digestion modification connection".

FlashCut™ Color Buffer includes red and yellow tracer dyes, which can be directly used for gel electrophoresis. The migration rate of red dye of FlashCut™ Color Buffer and 2500 bp double stranded DNA fragment in 1% agarose gel is similar; The migration rate of yellow dye and 10 bp double stranded DNA fragment in 1% agarose gel is similar.

Suggested reaction conditions

1. 1 × FlashCut™ Buffer solution.
2. Incubate at 37℃.
3. Prepare the reaction system according to the "DNA rapid enzyme digestion process".

Inactivation conditions

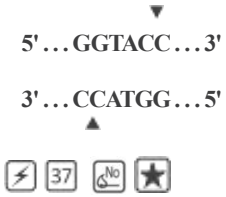
Cannot be thermally deactivated, please use phenol chloroform extraction or column purification.

Quality control

1. Functional activity detection: At 37℃, in a 20μL universal FlashCut™ reaction system, 1μL FlashCut™ KpnI can completely digest 1μg λ DNA(HindIII digest) within 15 minutes.
2. Long term incubation detection: At 37℃, 1μL FlashCut™ KpnI was incubated with 1μg λ DNA(HindIII digest) in a 20μL universal FlashCut™ reaction system for 3 hours, and no non-specific degradation of the substrate caused by other nuclease contamination or star activity was detected. Longer enzyme digestion may result in star activity.
3. Enzyme digestion ligation re digestion detection:At 37℃, use FlashCut™ KpnI with 10 times the enzyme amount digests DNA substrates, recovers enzyme cleavage products, and at 22℃, T4 DNA Ligase (Fast) can reconnect over 95% of the enzyme cleavage products. After recycling the connecting product again, more than 95% of the connecting product can be re cut using the same endonuclease.
4. Non specific endonuclease activity detection: At 37 ℃, 1μL of FlashCut™KpnI was dissolved in 20μL of the universal FlashCut™ reaction system and 1μg super spiral plasmid DNA were incubated together for 4 h, and then detected by agarose gel electrophoresis. Less than 10% of the plasmid DNA changed into a missing or linear state.
5. Blue white spot detection: Use 1μL FlashCut™KpnI to digest a specific vector containing the lacZ α gene and only having one enzyme cleavage site on that gene. Reconnect the enzyme digestion products and transform them into competent E. coli cells,

then coat them on LB agar containing X-gal, IPTG, and corresponding antibiotics for growth. The successfully connected β -galactosidase gene can be expressed correctly and grow blue colonies; Products that cannot be reconnected due to enzymatic degradation at the end will result in white colonies. For FlashCut™ series restriction enzymes, the proportion of white colonies should be less than 1%.

Icon annotation



Homolytic enzyme:Asp718I, Acc65I

Note: Homolytic enzymes may have different sensitivities to different methylation modifications.

1. Rapid endonuclease can complete the reaction within 5-15 minutes.
2. The optimal reaction temperature is 37℃.
3. Cannot be thermally deactivated.
4. 3 hours of incubation did not show star activity, and longer enzyme digestion may result in star activity.

Protocol

1. DNA rapid enzymatic digestion process

1.1. Prepare the reaction system on ice according to the recommended sample addition sequence as follows:

	plasmid DNA	PCR products	Genomic DNA
ddH_2O	15μL	16μL	30μL
10×FlashCut™ Buffer or 10×FlashCut™ Color Buffer	2μL	3μL	5μL
Substrate DNA	2μL(up to 1μg)	10μL(~0.2μg)	10μL(5μg)
FlashCut™ KpnI	1μL	1μL	5μL
Total	20μL	30μL	50μL

This system is suitable for enzyme digestion of purified PCR products. The unpurified PCR product has a certain ionic strength, 10×FlashCut ™ The amount of buffer added can be appropriately reduced to 2μL. However, due to the simultaneous exonuclease activity of DNA polymerase, it can affect the cleavage products. Therefore, the following steps require cloning and other operations. It is recommended to purify the PCR products before enzyme cleavage.

1.2. Gently suck or tap the tube wall to mix well (without vortexing), then centrifuge instantly to collect hanging wall droplets.

1.3. Incubate at 37℃ for 15 minutes (plasmid), or 15-30 minutes (PCR product), or 30-60 minutes (genomic DNA), it is not recommended to perform enzyme digestion for a long time.

1.4. Phenol chloroform extraction or column purification (optional).

1.5. If using FlashCut™ Color Buffer is used for enzyme digestion reaction, and the resulting product can be directly subjected to sample electrophoresis.

2. Double enzyme digestion or multi enzyme digestion

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 enzyme digestion, and then the enzyme with the higher optimal temperature

should be added for enzyme digestion reaction at its optimal reaction temperature.

3. Expansion reaction system suitable for plasmids

DNA	1μg	2μg	3μg	4μg	5μg
FlashCut™ KpnI	1μL	2μL	3μL	4μL	5μL
10×FlashCut™ Buffer or 10×FlashCut™ Color Buffer	1μL	2μL	3μL	4μL	5μL
Total	20μL	20μL	30μL	40μL	50μL

Note: If the total reaction system is greater than 20μL, the incubation time should be appropriately increased, and water bath, metal bath or sand bath should be used as much as possible.

The number of enzyme cleavage sites in different DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
2	0	0	1	1	1	1	8

The influence of methylation modification

Dam	Dcm	CpG	EcoKI	EcoBI
no effect	no effect	no effect	no effect	no effect

Activity in different reaction buffers

	FlashCut™ Buffer	Thermo Scientific FastDigest Buffer	NEB CutSmart® Buffer	Takara QuickCut™ Buffer
reactivity	100%	100%	100%	100%

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

The activity of DNA modifying enzymes in FlashCut™ Buffer and FlashCut™ Color Buffer

Alkaline Phosphatase (Fast)	100%
T4 DNA Ligase (Fast)	100%

Note: The activity data comes from the detection under standard reaction system, and T4 DNA Ligase (Fast) requires ATP as a cofactor.