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FlashCut™ SphI

Product Number: RE0571

Shipping and Storage

-20°C.

Components

| Component | Specifications |
|----------------------------|----------------|
| FlashCut™ SphI | 50μL |
| 10× FlashCut™ Buffer | 1mL |
| 10× FlashCut™ Color Buffer | 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°C.
3. Prepare the reaction system according to the "DNA rapid enzyme digestion process".

Inactivation conditions

Incubate at 80°C for 20 minutes.

Quality control

1. Functional activity detection: At 37°C, in a 20μL universal FlashCut™ reaction system, 1μL FlashCut™ SphI can completely digest 1μg λ DNA within 15 minutes.
2. Long term incubation detection: At 37°C, 1μL FlashCut™ SphI was incubated with 1μg λ DNA 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°C, use FlashCut™ SphI with 10 times the enzyme amount digests DNA substrates, recovers enzyme cleavage products, and at 22°C, 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. Detection of non-specific endonuclease activity: at 37°C, 1μL FlashCut™ SphI and 1μg super spiral plasmid DNA were incubated together for 4h in a 20μL universal FlashCut™ reaction system, and then detected by agarose gel electrophoresis, less than 10% of the plasmid DNA turned into a missing or linear state.
5. Blue white spot detection: Use 1μL FlashCut™ SphI 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,

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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: PaeI

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

1. ⚡ Rapid endonuclease can complete the reaction within 5-15 minutes.
2. 37 The optimal reaction temperature is 37°C.
3. EB For DNA methylated by EcoBI, splicing may be hindered
4. 20 80 Inactivation condition: Incubate at 80°C for 20 minutes.
5. ★ 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 ₂ O | 15μL | 16μL | 30μL |
| 10×FlashCut™ Buffer or 10×FlashCut™ Color Buffer | 2μL | 3μL | 5μL |
| Substrate DNA | 2μL(up to 1μg) | 10μL(up to~0.2μg) | 10μL(5μg) |
| FlashCut™ SphI | 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°C for 15 minutes (plasmid), or 15-30 minutes (PCR product), or 30-60 minutes (genomic DNA).

Note: Enzyme activity may slightly decrease during the reaction at 37°C, and the incubation time should be appropriately extended.

1.4. Incubate at 80°C for 20 minutes to inactivate the enzyme and stop the reaction (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™ SphI | 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 |
|------|-------|--------|-------|----------|------|------------|--------|
| 6 | 0 | 1 | 1 | 1 | 2 | 1 | 8 |

The influence of methylation modification

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

Activity in different reaction buffers

| Cut One™ | Thermo Scientific | NEB | Takara |
|------------|--------------------|-------------------|-------------------|
| Buffer | Fast Digest Buffer | Cut Smart® Buffer | Quick Cut™ Buffer |
| Reactivity | 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.