FspI

Introduction

FspI is a genetically engineered fast restriction endonuclide that is suitable for fast digestion of plasmid DNA, PCR products or genomic DNA, etc. (enzyme digestion is completed within 5-15 minutes). Its internal matching Reaction Buffer (Color) contains red and yellow tracer dyes for direct gel electrophoresis of the product. The migration rate of red dye and 2500 bp double-stranded DNA fragment in 1% agarose gel was similar, and the migration rate of yellow dye and 10 bp double-stranded DNA fragment in 1% agarose gel was similar.

Its recognition sequence and cutting site are as follows:

5'……TGC↓GCA……3'

3'……ACG↑CGT……5'

Components and Storage

Components \ Size	K3021 - 500 U	Storage
FspI (10 U/μL)	50 μL	-20℃
10×Reaction Buffer	1 mL	-20℃
10×Reaction Buffer (Color)	1 mL	-20℃
Shipping: Dry Ice	**Shelf life:** 2 years	

Protocol

I DNA Rapid enzyme digestion:

1. Refer to the table below to configure the reaction system on the ice:

Components	Plasmid DNA	PCR products	Genomic DNA
DNA substrate	X μL (1 μg)	X μL (~0.2 μg)	X μL (5 μg)
10×Reaction Buffer or 10×Reaction Buffer (Color)	2 μL	3 μL	5 μL
FspI	1 μL	1 μL	5 μL
Nuclease-free Water	To 20 μL	To 30 μL	To 50 μL
Total Reaction Volume	20 μL	30 μL	50 μL

Note: This system is suitable for enzymatic digestion of purified PCR products; Since DNase also has exonuclidene activity, it will affect the enzyme digestion products, so the following step needs to be cloned and other operations, it is recommended to purify the PCR products before enzyme digestion.

2. Gently mix the reaction system (gently suck or flick the tube wall, do not swirl), and then instantaneously centrifuge to collect the tube wall residue.
3. Incubate at 37℃ for 15 min (plasmid); 15-30 min (PCR product); 30-60 min (genomic DNA).
4. Phenol chloroform extraction or column purification (not heat inactivated) (optional).
5. If 10×Reaction Buffer (Color) is selected for enzyme digestion, the obtained product can be directly subjected to sample electrophoresis.

II Double or multiple enzyme digestion:

1. The dosage of each endonuclide is 1 μL, and the reaction system is appropriately expanded as needed.
2. The total volume of all endonuclease enzymes should not exceed 1/10 of the total reaction system.
3. If the optimal reaction temperature of several selected endonucleoenzymes is different, the enzyme with the lowest optimal temperature should be started first, and then the enzyme with the highest optimal temperature should be added to incubate at a higher temperature.

Notes

1. Add the FspI enzyme last when configuring the cleavage reaction system, and keep the enzyme on ice when removing it from the refrigerator.
2. If the total reaction system is greater than 20 μL, the incubation time should be appropriately increased, and the water bath, metal bath or sand bath should be used as far as possible.
3. Mix the components by blowing up and down the pipette or "flicking" the reaction tube, do not use violent vortex mixing.
4. FspI is not heat-inactivated and should be removed by phenol chloroform extraction or column purification. For DNA methylated by CpG, clipping may be blocked; In addition, asterisk activity may occur beyond 3 h incubation time.
5. For double or multi-enzyme digestion, an appropriate buffer compatible with two or more endonuclide enzymes should be selected, and the reaction system should be set up according to the table above. If there is no suitable buffer to choose from, one enzyme can be digested and purified first, and then another enzyme digestion reaction can be performed after purification.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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