QuickCut™ *Sph* I

GC|ATGC
CGTAC|G

Code No.: 1632
Size: 50 µl (50 reactions)

Supplied Reagents:

10X QuickCut Buffer	500 µl
10X QuickCut Green Buffer	500 µl

Description:

QuickCut restriction enzyme is a kind of enzyme which can quickly digest substrate DNA. The digestion activity of each QuickCut enzyme can reach 100% in the 10X QuickCut Buffer or 10X QuickCut Green Buffer, and the substrate DNA, such as plasmid DNA and PCR product, can be cut completely in 5 to 30 min. In addition, 10X QuickCut Buffer and 10X QuickCut Green Buffer are available for other enzymes also in QuickCut series. Then some kinds of the QuickCut enzymes can be reacted simultaneously in one reaction.

So QuickCut enzyme can lead easy operation, time saving, and elimination of the complex operation in restriction enzyme digestion.

Each QuickCut enzyme is supplied together with two kinds of universal buffer: 10X QuickCut Buffer and 10X QuickCut Green Buffer. 10X QuickCut Green Buffer contains composition of the sample loading buffer for agarose gel electrophoresis in 10X QuickCut Buffer, so the digested DNA can be used for electrophoresis directly. This buffer contains two tracking dyes and the migration speed of the blue dye is equivalent to that of 3–5 kb DNA fragment and the yellow dye is to 10 bp DNA fragment in 1% agarose gel.

Storage Buffer:

10 mM	Tris–HCl, pH7.5
100 mM	KCl
0.1 mM	EDTA
1 mM	DTT
0.01%	BSA
0.15%	TritonX–100
50%	Glycerol

Storage : –20℃

Source: *Streptomyces phaeochromogenes*

Protocol:

1. Prepare reaction mixture in accordance with the table below:

	Linear DNA	Plasmid DNA	PCR product
10X QuickCut Buffer* or 10X QuickCut Green Buffer*	1 µl–5 µl	1 µl–5 µl	1 µl–3 µl
DNA	⩽ 1 µg	⩽ 1 µg	⩽ 0.2 µg
QuickCut *Sph* I	1 µl	1 µl	1 µl
Sterilized water	up to 10 µl–50 µl	up to 10 µl–50 µl	up to 10 µl–30 µl

*: With different reaction system, the amount of 10X Buffer is different. Please make sure the final concentration is 1X.

2. Mix gently and centrifuge quickly.
3. Incubate at 37℃ for 5～10 min*.
   *: 5 min for linear DNA. 10 min for plasmid DNA. 5 min for PCR product.

Activity Assay:

1 µg of λ DNA could be completely digested after incubation of 1 µl of QuickCut enzyme with 1X QuickCut Buffer or 1X QuickCut Green Buffer in 50 µl of reaction mixture at 37℃ for 5 min.

Quality Control:

1) Functional Activity Test:
   1 µg of linear DNA could be completely digested after incubation with 1 µl of QuickCut enzyme in 50 µl of reaction mixture at 37℃ for 5 min.
2) Prolonged incubation test:
   Non–specific nuclease activity was not detected when 1 µg of linear DNA was incubated with 1 µl of QuickCut enzyme for 16 hr in 50 µl of 1X QuickCut Buffer or 1X QuickCut Green Buffer at 37 ℃ as evaluated by agarose gel electrophoresis.
3) Exonuclease contamination test:
   After incubation of 1 µg of substrate with an amount of enzyme equivalent to that contained in 1 µl of this product for 30 minutes, specific DNA fragments were ligated with T4 DNA ligase, and no exonuclease activity was detected.

Effect of DNA methylation:

This enzyme is not influenced by CG methylase.

Note:

1) For double or multiple digestion, the amount of enzymes should not exceed 1/10 of the total reaction volume.
   If the reaction temperatures are different from each other, it is recommended that first, the lower temperature reaction should be performed with only specific enzyme which require lower temperature, and then add other enzyme which require higher temperature and perform second reaction.
2) 10X QuickCut Green Buffer may interfere with the fluorescence analysis. So, in the case to perform the fluorescence analysis of the digested product, the use of 10X QuickCut Buffer is recommended.
3) If precipitation appears in 10X QuickCut Green Buffer, dissolve completely by vortexing for 5 minutes at room temperature, which does not affect the quality.

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Note
This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.
Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from Takara Bio Inc.
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