QuickCut™ *Pvu* I

C G A T C G
G C T A G C

Code No.: 1625
Size: 25 μl (25 reactions)

Shipping at: −20℃
Store at: −20℃

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, pH8.0
100 mM	KCl
0.1 mM	EDTA
1 mM	DTT
0.01%	BSA
0.15%	TritonX–100
50%	Glycerol

Source: *Proteus vulgaris*

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 *Pvu* 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 5～15 min*.
   *: 5 min for linear DNA. 15 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) Star Activity Test:
   After incubation of 1 μg of DNA with 1 μl of QuickCut enzyme for 16 hours, no change of DNA bands pattern could be observed in agarose gel electrophoresis.
3) Labeled Oligonucleotide Assay (LOA) Test:
   After incubation of oligonucleotide labeled with fluorescence with 1 μl of QuickCut enzyme at 37℃ for 1 hour, the decomposition rate should be less than 10%.

Effects of DNA methylation:

Enzyme activity is affected by CG methylase, but not affected by dam methylase.

Note:

1) Ligation efficiency of DNA fragments with cohesive end generated by this enzyme is lower than others. Therefore, more efficient ligation can be achieved by using the reaction conditions for blunt end ligation
2) To avoid star activity, the reaction time should be less than 16 hours.
3) 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.
4) 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.
5) 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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