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MinuteCut™ Taq I Instructions

Digestion Site

5'...T▼CGA...3'

3'...AGC▲T...5'

Composition

MinuteCut™ Taq I	10 Preps	200 Preps
Cat. No.	6046010	6046200
MinuteCut™ Taq I	10 µl	200 µl
10×MinuteCut™ Buffer	50 µl	1.5 ml
10×MinuteCut™ Red Buffer	50 µl	1.5 ml
10×Loading Buffer	1.5 ml	1.5 ml
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Storage

This product can be stored at -20℃ for up to 2 years.

Technical Support

R&D Department, Hangzhou Simgen Biotechnology Co., Ltd. E-mail: technical@simgen.cn, Tel: 400-0099-857.

Introduction

MinuteCut™ enzymes are a series of engineered restriction enzymes that are capable of fast DNA digestion. All MinuteCut™ enzymes show superior activity in the universal 10×MinuteCut™ Buffer and 10×MinuteCut™ Red Buffer and are able to digest DNA in 5~15 min. This enables any combination of restriction enzymes to work simultaneously in one reaction tube and eliminates the need for sequential digestions. MinuteCut™ enzymes have passed multiple strict quality controls, and can be used to digest plasmid, genomic and viral DNA as well as PCR products. 10×MinuteCut™ Red Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the 10×MinuteCut™ Red Buffer migrates with 2.5 kb double-strand DNA fragments in a 1% agarose gel, and the yellow dye migrates with 10 bp double-strand DNA fragments in a 1% agarose gel.

Quality Control

Functional Test

A 20 µl reaction in 10×MinuteCut™ Buffer containing 1 µg of λDNA and 1 µl of MinuteCut™ Taq I incubated for 15 min at 37℃ results in complete digestion as determined by agarose gel electrophoresis.

Prolonged Incubation/Star Activity Assay

A 20 µl reaction in 10×MinuteCut™ Buffer containing 1 µg of λDNA and 1 µl of MinuteCut™ Taq I incubated for 3 hours at 37℃ results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. Longer incubation may result in star activity.

Ligation and Recutting

After 10-fold over-digestion with MinuteCut™ Taq I at 37℃, >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃. Of these ligated fragments, >95% can be recut with MinuteCut™ Taq I as determined by agarose gel electrophoresis.

Icon Descriptions

- This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.
- The enzyme's optimum reaction temperature is 65℃.
- The enzyme can not be thermal inactivated.
- Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the Dam methylase.
- 3 hours incubation do not show star activity, but longer incubation may result in star activity

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Protocol

1. Rapid digestion of DNA

Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	*PCR Product	Genomic DNA
DNA	≤1μg	≤ 0.2μg	≤1μg
MinuteCut™ Taq I	1 μl	1 μl	1 μl
10×MinuteCut™ Buffer or 10×MinuteCut™ Red Buffer	2 μl	3 μl	3 μl
ddH_2O	5~15 μl	5~20 μl	5~20 μl
Total	20 μl	30 μl	30 μl

* This system is suitable for digestion of purified PCR products. The unpurified PCR product has metal ions, and because of the exonuclease activity of some DNA polymerases may alter the end of cleaved DNA. So, if the digestion product will be used for cloning , it is recommended to use the Simgen DNA Purification Kit (Cat. No. 2101050) to purify the PCR product before digestion.

(1) Mix gently and spin down.

(2) Incubate at 37°C for 5 min (PCR product and genomic DNA) or for 15 min (plasmid).

(3) Optional: Phenol/chloroform treatment or column based purification.

For restriction enzymes that cannot be heat inactivated, it is recommended to use 10× MinuteCutTM Buffer for digestion, and then mix with 10×Loading Buffer（containing SDS）for electrophoresis, otherwise the enzyme digestion product may appear smeared in electrophoresis.

(4) If the 10×MinuteCut™ Red Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel. If the 10×MinuteCut™ Buffer was used in the reaction, mixed with 10×Loading Buffer, then load an aliquot of the mixture on a gel.

2. Double and Multiple Digestion of DNA

(1) Use 1 μl of each enzyme and scale up the reaction conditions appropriately.

(2) The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume. e.g., the commonly used double digestion system needs to be digested in at least 20 μl reaction system.

(3) If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.

Precautions

1) It is not recommended to incubate more than 10 h, longer incubation may result in star activity.

2) 10×MinuteCut™ Red Buffer may interfere with the fluorescence analysis of enzyme digestion products. Therefore, a colorless 10×MinuteCut™ Buffer is recommended for fluorescence analysis of enzyme digest products.

3) If the DNA digestion cannot be cut after 5 min, it means that the DNA contains inhibitors that inhibit the activity of restriction enzyme, and the Simgen DNA purification kit (Cat. No. 2101050) can be used to purify the DNA and then perform the enzyme digestion.

4) If the plasmid DNA extracted from wild-type E. coli or non-restriction endonuclease (endA+) host bacteria (such as BL21, HB101, etc.) is used for digestion, the digestion product may be smeared band after electrophoresis, and Simgen DNA purification kit (Cat. No. 2101050) can be used for plasmid DNA purification and then digested.