Bsa I (RNase-free)

Product description

This product BsaI is a protein mutant encoded by the Bsa I gene of Bacillus stearothermophilus (Bacillus stearothermophilus) expressed by recombinant E.coli. It is an IIS-class restriction enzyme that is often used to linearize plasmid synthesis of mRNA vaccines or other in vitro transcriptional plasmids.

Its identification sequence and cutting site are as follows:

5'…… GGTCTC(N)↓…… 3'

3'…… CCAGAG(NNNNN)↑…… 5'

Composition and storage conditions

Components \ Size	1000U	5000 U	10 KU
Bsa I (RNase-free)	0.05 mL	0.25 mL	0.5 mL
10×Cut^{rA} Buffer	0.1 mL	0.5 mL	1 mL
Store the components at -20 °C.			

Experimental operation

I Rapid DNA digestion process:

1. Refer to the following table to add the reaction system to the ice operation:

Reagent	Plasmid DNA
DNA	~1 μg
10×Cut^{rA} Buffer	2 μL
Bsa I	1 μL
Nuclease-free Water	to 20 μL

2. Gently stroke or flick the wall of the tube to mix well (never vortex) and then instantaneously centrifuge to collect the wall-mounted droplets.

3. Incubate at 37 °C for 60 min.

4. Incubate at 80 °C for 20 min to inactivate the enzyme and stop the reaction (optional).

II Double or multi-digestion:

1. The amount of each endonuclease is 1 μL and the reaction system is appropriately expanded as needed.
2. The sum of the volumes of all endonucleases should not exceed 1/10 of the total reaction system.
3. If the optimal reaction temperature of several endonucleases selected is different, the digestion should be started with the enzyme with the lowest optimal temperature, and then the enzyme with the higher optimal temperature should be added to incubate at a higher temperature.

The nature of the product

1. **Enzyme Viable Units:** At 37 °C, the amount of enzyme required to completely cleave 1 μg of internal control DNA (containing a BsaI resection site) within 1 h is defined as 1 active unit.
2. **Store the solution:** 10 mM Tris-HCl(pH 7.4 @ 25°C)，200 mM NaCl，1 mM DTT，0.1 mM EDTA，200 μg/ml Recombinant Albumin，50% Glycerol.
3. **10×Cut^{rA} buffer:** 500 mM Potassium Acetate，200 mM Tris-acetate(pH 7.9 @ 25°C)，100 mM Magnesium Acetate，1 mg/ml Recombinant Albumin.

Quality assurance

1. Protein Purity Test: SDS-PAGE Test Purity > 95%.
2. Functional activity detection: At a reaction temperature of 37 °C, in a 50 μL reaction system, 50 U enzymes are capable of completely digesting 50 μg of pcDNA 3.1-BsaI-DNA within 1 h.
3. Non-specific nuclease detection: At 37 °C reaction temperature, 20U of enzyme will be incubated with pcDNA 3.1-BsaI-DNA at 37 °C for 14 h, no non-specific degradation of substrates caused by other nuclease contamination or asterisk activity is detected, but asterisk activity may occur if the incubation time is prolonged.
4. Digestion-ligation-re-digestion detection: The enzyme completely digests the substrate, recovers the digestion product, re-ligates the digestion product using an appropriate amount of T4 DNA ligase at 16 °C, and after the ligation product is recovered again, the ligation product can be re-cut using the same endonuclease.
5. Nucleic acid endonuclease activity detection: At 37 °C reaction temperature, after incubating 20U enzyme with 1 μg of superhelix 174 DNA for 4 h, using agarose gel electrophoresis detection, plasmid DNA lysis superhelier < 10%.
6. Detection of exonuclease activity: 20U of enzyme and Hhind III- DNA were incubated at 37 °C for 16 h, the electrophoresis band did not change, and there was no non-specific exonuclease activity.
7. RNase detection: 20 U of this enzyme and 200 ng of 300 base RNA substrate reacted at 37 °C for 4 h, and the electrophoresis band of RNA did not change.
8. Heat inactivation conditions: Heating at 80 °C for 20 minutes can inactivate enzymes.

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