Product Specification

Product Name:	SpeedyCut *BsaI* Restriction Enzyme
Product Code:	SGRE04700

Product Description:

BsaI is categorized as a SpeedyCut series Restriction Enzymes (RE). It is a subset of RE that are capable of cutting DNA within 5 – 15 minutes. All SpeedyCut RE are compatible with the universal SpeedyOne buffer, this universal buffer allows for combination of different enzymes within a standardized reaction system without the need for multiple rounds of single enzyme cuts. They are convenient, stable, fast and can accurately cut DNA.

BsaI is a type of Type II class RE. The SpeedyCut series RE undergoes strict quality control processes to ensure its reliability for rapid digestion of plasmid DNA, genomic DNA and PCR products.

***BsaI* Recognition Site**
5'…-GGTCTC(N)1↓…-3' 3'…-CCAGAG(N)5↑…-5'

Product Content(s)

SpeedyCut *BsaI* RE	50µl
10X SpeedyOne buffer	1ml

Storage: -20ºC

Note: At 37ºC, in a 20µl reaction system, 1µl enzyme can completely digest 1µg pPIC9K DNA in 15 minutes. If there's a difference in the optimal temperatures of the chosen enzymes, consider starting the digestion reaction with the enzyme having the lower optimal temperature, and then subsequently add the enzyme with the higher optimal temperature.

Procedures:

1. Recommended DNA digestion process

Plasmid DNA

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	2µl
DNA	2µl (up to 1µg)
SpeedyCut *BsaI*	1µl
Nuclease – free water	15µl
Total	20µl

Note: The volume of all enzyme added should not exceed 10% of the total volume to prevent an excess of glycerol in the enzyme, which may lead to star activity.

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PCR Product

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	3µl
DNA	10µl (0.2µg)
SpeedyCut *BsaI*	1µl
Nuclease – free water	16µl
Total	30µl

Genomic DNA

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	5µl
DNA	10µl (5µg)
SpeedyCut *BsaI*	5µl
Nuclease – free water	30µl
Total	50µl

Note: The above three systems are suitable for enzymatic digestion of purified PCR products. In case of unpurified PCR products, it contains a certain ion strength, the 10X SpeedyOne buffer added can be reduced to 2µl. However, due to the concurrent exonuclease activity of DNA polymerase, it is highly advised to purify the PCR products before proceeding with enzymatic digestion.

b. Pipette all reagents into a microcentrifuge tube (according to the tables above) and gently resuspend to ensure it is homogenized (Do not Vortex). Immediately centrifuge the tubes to ensure no reagents are left on the side of the tube.
c. Incubate at 37°C for 15 minutes for plasmid DNA ,15 – 30 minutes for PCR products or 30 – 60 minutes for genomic DNA.
d. Incubate at 80°C for 20 minutes to inactivate enzyme and stop the reaction (optional). Alternatively, purification method like spin column or phenol / chloroform extraction could be used to stop the reaction.

2. Scaling up Plasmid DNA Digestion Reaction

Reagents	Volume				
DNA	1µg	2µg	3µg	4µg	5µg
10X SpeedyOne Buffer	2µl	2µl	3µl	4µl	5µl
SpeedyCut *BsaI*	1µl	2µl	3µl	4µl	5µl
Nuclease – free water	Top up till 20µl	Top up till 20µl	Top up till 30µl	Top up till 40µl	Top up till 50µl
Total	20µl	20µl	30µl	40µl	50µl

Note: If the total reaction exceeds 20µl, it is advisable to extend the incubation time accordingly and utilize water bath, sand bath and heat block.

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Enzyme properties

1. Methylation Effects

a. Dam: Never overlap – no effects
b. Dcm: Overlap – digestion is hindered
c. CpG: Overlap – digestion is hindered
d. *EcoKI*: Never overlap – no effects
e. *EcoBI*: Never overlap – no effects

2.Heat Inactivation

a. Heat inactivated at 80 °C for 20 minutes

3. Number of recognition sites in DNA

a. λDNA – 2
b. φX174 - 0
c. M13mp18/19 - 0
d. pBR322 – 1
e. pUC57 – 1
f. pUC18/19 – 1
g. pACYC184 – 0
h. Adeno2 – 18

Isochizomers

a. Eco31I, Bso31I, BspTNI

*Isochiomers may have different sensitivity to different methylation modification

Note:

This product is designed for research use only. Not to be used on humans or animals' diagnosis and other in vivo experiments.

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