BsaI

Cat. No.: L359050 | Pack size: 1 KU; 5 × 1 KU | Storage: -20 °C

Overview

Restriction endonucleases, abbreviated as restriction enzymes, are a class of endodeoxyribonucleases that recognize specific deoxyoligonucleotide sequences and cleave the phosphodiester bond between two deoxyribonucleotides at a specific position on each DNA strand. BsaI is a Type IIS restriction enzyme; it recognizes non-palindromic sequences and cuts outside the recognition sequence to generate sticky overhangs.

Isoschizomers: Eco31I, Bso31I, BspTNI

Recognition Site: GGTCTC(1/5)

5'...G G T C T C $(N)_1$ ↓...3'

3'...C C A G A G $(N)_5$ ↑...5'

Components List

L359050	Component	Appearance	1KU	5×1KU	Storage
L359050A	BsaI (20U/μl)	Liquid	50μl	5×50μl	-20℃
L359050B	10×Buffer	Liquid	1ml	5×1ml	-20℃
L359050C	10×Color Buffer	Liquid	1ml	5×1ml	-20℃

Recommended Reaction Conditions

1× Buffer;

Incubate at 37 °C;

Refer to "Protocol for Fast DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Quality Control

Functional Test

A 20 µl reaction in Buffer containing 1 µg of pPIC9K DNA and 1 µl of BsaI incubated for 15 minutes at 37℃ results in complete digestion as determined by agarose gel electrophoresis.

Prolonged Incubation / Star Activity Assay

A 20 µl reaction in Buffer containing 1 µg of pPIC9K DNA and 1 µl of BsaI 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 Recuting

After 10-fold over-digestion with BsaI 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 BsaI as determined by agarose gel electrophoresis.

Non-specific Endonuclease

Activity A 20 µl reaction in Buffer containing 1 µg of supercoiled plasmid and 1 µl of BsaI incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Method of application

1. Protocol for Fast DNA Digestion

① Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	PCR Product	Genomic DNA
ddH_2O	15 µl	16 µl	30 µl
10×Buffer or 10×Color Buffer	2 µl	3 µl[a]	5 µl
DNA	2 µl (up to 1 µg)	10 µl (~0.2 µg)	10 µl (5 µg)
BsaI (20U/µl)	1 µl	1 µl	5 µl

	Plasmid DNA	PCR Product	Genomic DNA
Total	20 µl	30 µl	50 µl

a. For purified PCR products. If the PCR products are not purified, amount of 10× Buffer should be reduced to 2 µl due to the remaining metal ions in the unpurified PCR products. We recommend to purify PCR products before digestion if it will be used for cloning, because the exonuclease activity of some DNA polymerases may alter the end of cleaved DNA.

② Mix gently and spin down;

③ Incubate at 37°C for 15 minutes (plasmid DNA) or for 15~30 minutes (PCR product) or for 30~60 minutes (genomic DNA);

④ Optional: Inactivate the enzyme by heating for 20 minutes at 80°C;

⑤ If the Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly 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;

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.

3. Scaling up Plasmid DNA Digestion Reaction

	1 µg DNA	2 µg DNA	3 µg DNA	4 µg DNA	5 µg DNA
DNA	1 µg	2 µg	3 µg	4 µg	5 µg
BsaI (20U/µl)	1 µl	2 µl	3 µl	4 µl	5 µl
10×Buffer or 10×Color Buffer	2 µl	2 µl	3 µl	4 µl	5 µl
Total	20 µl	20 µl	30 µl	40 µl	50 µl

Note: Increase the incubation time if the total reaction volume exceeds 20 µl.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18 /19	Adexnc2
2	0	1	1	1	0	0	18

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	Impaired	Impaired	No effect	No effect

Specifications

Attribute	Value
Product Name	BsaI
Synonyms	Eco31I | BsaI | Bsa I | Eco31I | Bso31I | BspTNI
Specifications & Purity	Recombinant, for DNA synthesis, Suitable for molecular biology, EnzymoPure™, For In Vitro Transcription, for DNA and RNA applications, 20 U/µl; expressed in E.coli
Bioactivity	20 U/µl
Molecule Type	Enzyme

Storage and Shipping

Attribute	Value
Concentration	20 U/µl; expressed in E.coli
Storage	Store at -20°C,Avoid repeated freezing and thawing
Shipped In	Ice chest + Ice pads
Stability And Storage	Store at 2-8℃ short term (1-2 weeks). Store at -20℃ long term (24 months). Upon receipt, it is recommended to aliquot.

Attribute	Value
Unit definition	One unit (U) is defined as the amount of enzyme required to completely digest 1 µg of pPIC9K (Dcm^-) DNA in a 50 µL reaction system at 37 °C within 1 hour.

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