FlashDigest BsaI

50 rxn

Cat No: FD0002

Shipping : Ship with dry ice.
Storage : Store at -20°C.

General Information

FlashDigest Fast Endonucleases are a series of genetically engineered restriction enzymes designed for fast and efficient digestion of plasmid DNA, PCR products, and genomic DNA. *FlashDigest* enzymes exhibit high activity in both 10x *FlashDigest* Buffer and 10x *FlashDigest* Red Buffer, enabling complete digestion within 5–15 minutes.

10x *FlashDigest* Buffers ensure 100% activity for all *FlashDigest* enzymes and is optimized for efficient double digestion reactions, enabling reliable and streamlined workflows in a single tube.

10x *FlashDigest* Red Buffer contains optimized red and yellow tracking dyes, allowing direct loading onto agarose gels without the need for additional loading dye. In a 1% agarose gel, the red dye migrates similarly to a 2500 bp double-stranded DNA fragment, while the yellow dye migrates comparably to a 10 bp fragment.

Restriction Site

Protocol and Reaction Conditions

1. Prepare the reaction mixture on ice according to the recommended reaction conditions as outlined below:

Components	Plasmid DNA	PCR Products*	Genomic DNA
DNA	up to 1 µg	up to ~0.2 µg	5 µg
10x *FlashDigest* Buffer and 10x *FlashDigest* Red Buffer**	2 µl	3 µl	5 µl
FlashDigest BsaI	1 µl	1 µl	5 µl
Nuclease Free Water	to 20 µl	to 30 µl	to 50 µl
Total	20 µl	30 µl	50 µl

*Unpurified PCR products may contain residual salts and enzymes that affect digestion efficiency. If used directly, the volume of 10x *FlashDigest* Buffer or 10x *FlashDigest* Red Buffer can be reduced to 2 µL. However, for optimal results in downstream applications such as cloning, purification of PCR products prior to digestion is strongly recommended.

**10x *FlashDigest* Buffer and 10x *FlashDigest* Red Buffer is fully compatible with Thermo Scientific FastDigest Buffer, NEB CutSmart® Buffer, and Takara QuickCut™ Buffer, providing 100% enzymatic activity across all systems.

2. Mix gently by pipetting or tapping the tube (do not vortex), then briefly centrifuge to collect any droplets from the tube walls.

3. Incubate at 37°C for 15 minutes (plasmid DNA), 15–30 minutes (PCR products), or 30–60 minutes (genomic DNA).

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

5. When using *FlashDigest* Red Buffer, the digestion products can be directly loaded onto an agarose gel for electrophoresis.

For further information;
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Double or Multi Enzyme Digestion

1. Use 1 µL of each *FlashDigest* endonuclease per reaction; adjust the total reaction volume proportionally if needed.

2. The combined volume of all rapid endonucleases should not exceed 10% of the total reaction volume.

Important Notes

- 10x *FlashDigest* Buffer and 10x *FlashDigest* Red Buffer support 100% activity of DNA modifying enzymes, including Fast Alkaline Phosphatase and Fast T4 DNA Ligase. Note that Fast T4 DNA Ligases require ATP as a cofactor.

- The number of enzyme cleavage sites of *FlashDigest* BsaI in different DNA substrates is as follows:

DNA Substrate	λ DNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
Cleavage Sites	2	0	1	1	1	0	1	18

Quality Control

Functional Activity Test:
At 37°C, 1 µL of *FlashDigest* BsaI completely digests 1 µg of pPIC9K within 15 minutes in a 20 µL universal 10x *FlashDigest* reaction system.

Prolonged Incubation Test:
At 37°C, 1 µL of *FlashDigest* BsaI was incubated with 1 µg of pPIC9K in a 20 µL universal *FlashDigest* reaction system for 3 hours. No non-specific degradation due to nuclease contamination or star activity was observed. However, extended incubation may increase the risk of star activity.

Digestion–Ligation–Re-digestion Test:
At 37°C, DNA substrates were digested using *FlashDigest* BsaI at 10× enzyme concentration. The digestion products were purified and subsequently ligated at 22°C using Fast T4 DNA Ligase, achieving over 95% ligation efficiency. Upon re-digestion with the same enzyme, more than 95% of the ligated products were successfully cleaved again.

Non-specific Endonuclease Activity Test:
At 37°C, 1 µL of *FlashDigest* BsaI was incubated with 1 µg of supercoiled plasmid DNA in a 20 µL universal *FlashDigest* reaction system for 4 hours. Agarose gel electrophoresis analysis showed that less than 10% of the plasmid DNA converted to nicked or linear forms.

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