

FlashDigest EcoRI

600 rxn

Cat No: FD0001

Shipping : Ship with blue/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 <i>FlashDigest</i> Buffer and 10x <i>FlashDigest</i> Red Buffer**	2 µl	3 µl	5 µl
<i>FlashDigest</i> EcoRI	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.

- Mix gently by pipetting or tapping the tube (do not vortex), then briefly centrifuge to collect any droplets from the tube walls.
- Incubate at 37°C for 15 minutes (plasmid DNA), 15–30 minutes (PCR products), or 30–60 minutes (genomic DNA).
- Incubate at 80°C for 20 minutes to inactivate the enzyme and terminate the reaction (optional).
- When using *FlashDigest* Red Buffer, the digestion products can be directly loaded onto an agarose gel for electrophoresis.

Double or Multi Enzyme Digestion

1. Use 1 μ L 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* EcoRI in different DNA substrates is as follows:

DNA Substrate	λ DNA	Φ X174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
Cleavage Sites	5	0	1	1	1	1	1	5

Quality Control

Functional Activity Test:

At 37°C, 1 μ L of *FlashDigest* EcoRI completely digests 1 μ g of λ DNA within 15 minutes in a 20 μ L universal 10x *FlashDigest* reaction system.

Prolonged Incubation Test:

At 37°C, 1 μ L of *FlashDigest* EcoRI was incubated with 1 μ g of λ DNA in a 20 μ L 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* EcoRI at 10 $\times$ 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 μ L of *FlashDigest* EcoRI was incubated with 1 μ g of supercoiled plasmid DNA in a 20 μ L 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.