# *FlashDigest* EcoRI

600 rxn

**Cat No:** FD0001

**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* 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.

**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.

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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* EcoRI in different DNA substrates is as follows:

| DNA Substrate | λ DNA | ΦX174 | 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 µL of *FlashDigest* EcoRI completely digests 1 µg of λ DNA within 15 minutes in a 20 µL universal 10x *FlashDigest* reaction system.

**Prolonged Incubation Test:**
At 37°C, 1 µL of *FlashDigest* EcoRI was incubated with 1 µg of λ DNA 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* EcoRI 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* EcoRI 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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