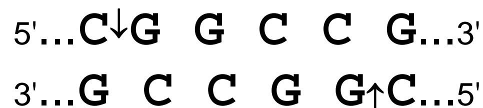
Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

FastDigest™ EagI (Eco52I)*

#FD0334 50 µl (for 50 reactions)

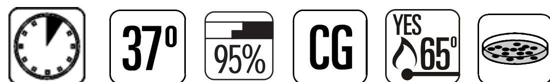
Lot: **Expiry Date:**



* FastDigest™ EagI (Eco52I) is a proprietary formulation of Eco52I, an isoschizomer of EagI having the same recognition and cleavage specificity.

Supplied with: 1 ml of 10X FastDigest™ Buffer

Store at -20°C



In total 2 vials. BSA included: Lot# BSA62-313P

ISO 9001 | ISO 14001
www.fermentas.com

Description

FastDigest™ enzymes are an innovative formulation of Fermentas restriction enzymes for target DNA digestion in only 5 minutes. All FastDigest™ enzymes work in the same buffer, which permits convenient and rapid double and multiple DNA digestions.

FastDigest™ enzymes are conveniently formulated: 1 µl of enzyme can completely digest up to 1 µg of DNA.

Features

- All FastDigest™ enzymes work in the same reaction conditions
- Single and double digestion of DNA in only 5 min
- No star activity in prolonged incubations
- Enhanced performance in one-hour DNA cleavage reactions

Visit www.fermentas.com for an updated list of FastDigest™ enzymes and protocols related to their use.

ENZYME PROPERTIES

Unit Definition

One FastDigest™ Unit (FDU) is the amount of the enzyme required to cleave 1 µg of lambda DNA-Eco81I fragments in 5 min at 37°C in 1X FastDigest™ Buffer.

Concentration

1 FDU/µl

Recommended Reaction Conditions

1X FastDigest™ Buffer
Incubation at 37°C

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: completely overlaps – blocked.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Compatible Ends

Bsp120I, CfrI, NotI

Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
2	0	1	0	0	0	0

Thermal Inactivation

FastDigest™ EagI (Eco52I) is inactivated by incubation at 65°C for 5 min.

Digestion of Plasmid DNA

1 µl of FastDigest™ EagI (Eco52I) digests up to 1 µg of plasmid DNA in 20 min.

Digestion of PCR Products

1 µl of FastDigest™ EagI (Eco52I) digests ~0.2 µg of PCR product in 20 min.

Digestion of Genomic DNA

1 µl of FastDigest™ EagI (Eco52I) digests 1 µg of genomic DNA in 5 min, or 5 µg of genomic DNA in 30 min.

QUALITY CONTROL ASSAY DATA

Functional Activity Test

1 µg of lambda DNA-Eco81I fragments was completely digested with 1µl of the enzyme in 5 minutes at 37°C in 20 µl of reaction mixture.

Ligation/Recutting Assay

After overdigestion with 1 µl of FastDigest™ EagI (Eco52I) for 1 hour, more than 95% of DNA fragments can be ligated and recut.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded oligonucleotides occured during incubation with 1 µl of FastDigest™ EagI (Eco52I) for 1 hour.

Prolonged Incubation / Star Activity Assay

No detectable degradation of 1 µg of lambda DNA due to nuclease contamination or star activity occurred during incubation with 1 µl of FastDigest™ EagI (Eco52I) for 16 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 1 µl of FastDigest™ EagI (Eco52I) for 16 hours. After religation and transformation, the background level of white colonies was 0.3%.

Quality authorized by: Jurgita Zilinskiene

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Protocol for Fast Digestion of DNA

❶ Combine the following reaction components at room temperature in the order indicated:

	Plasmid DNA	PCR product	Genomic DNA
Water*, nuclease-free (#R0581)	15 µl	17 µl	30 µl
10X FastDigest™ buffer	2 µl	2 µl	5 µl
DNA*	2 µl (up to 1 µg)	10 µl (~0.2 µg)	10 µl (5 µg)
FastDigest™ enzyme	1 µl	1 µl	5 µl
Total volume:	20 µl	30 µl	50 µl

❷ Mix gently and spin down.

❸ Incubate at 37°C in a heat block or water thermostat for 5 min (genomic DNA), or for 20 min (PCR product and plasmid DNA).

❹ Inactivate the enzyme by heating for 5 min at 65°C (optional).

Double and Multiple Digestion of DNA

FastDigest™ enzymes allow simultaneous digestion of DNA with two or more enzymes in one digestion reaction.

- Use 1 µl of each enzyme and scale up the reaction conditions appropriately.
- The combined volume of all added enzymes should not exceed 1/10 of the total reaction volume.

Reaction Set-up for Digestion of Multiple DNA Samples

❶ Pipette 2 µl of DNA* samples into tubes

❷ Prepare a master mix for n+1 samples

Example of master mix (for 10 samples of plasmid DNA):

Water*, nuclease-free (#R0581)	(10+1) x 15 µl = 165 µl
10X FastDigest™ buffer	(10+1) x 2 µl = 22 µl
FastDigest™ enzyme	(10+1) x 1 µl = 11 µl

❸ Add 18µl of master mix* into tubes containing DNA.

* The volume of DNA can be scaled up to 10 µl or down to 0.5 µl depending on the DNA concentration. The volume of water and master mix should be corrected to keep the indicated total reaction volume.

Scaling up DNA Digestion Reaction

DNA	1 µg	2 µg	3 µg	4 µg	5 µg
FastDigest™ enzyme	1 µl	2 µl	3 µl	4 µl	5 µl
10X FastDigest™ buffer	2 µl	2 µl	3 µl	4 µl	5 µl
Total volume:	20 µl	20 µl	30 µl	40 µl	50 µl

Important Notes

- Always check the sensitivity of enzyme to DNA methylation (*see* **Methylation Effects on Digestion**).
- The context of the target sequence may affect DNA cleavage efficiency. Prolonged incubation time is recommended to achieve complete digestion.
- PCR additives such as DMSO or glycerol may affect the cleavage efficiency or cause star activity.
- When introducing restriction enzyme sites into primers for subsequent digestion and cloning of a PCR product, refer to the Table "Cleavage efficiency close to the termini of PCR fragments" (www.fermentas.com) to define the number of extra bases required for efficient cleavage.
- For cloning applications, purification of PCR products prior to digestion is highly recommended to remove the active thermophilic DNA polymerase still present in PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield.
- Increase the incubation time by 3-5 min if total reaction volume exceeds 20 µl. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively *for research purposes and in vitro use only.* The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

(1) Revised 30.08.2007