

CERTIFICATE OF ANALYSIS

FastDigest™ AlwNI (CaiI)*

#FD1394 50µl (for 50 reactions)

Lot: **Expiry Date:**

5'...**C A G N N N↓C T G**...3'
3'...**G T C↑N N N G A C**...5'

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

Supplied with: 1ml of 10X FastDigest™ Buffer

Store at -20°C



In total 2 vials.

BSA included: Lot# BSA62-313P

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: 1µl of enzyme can completely digest up to 1µg of DNA.

Features

- All FastDigest™ enzymes work in the same reaction conditions
- Single and double digestion of DNA in only 5min
- 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 1µg of lambda DNA in 5min at 37°C in 1X FastDigest™ Buffer.

Concentration

1FDU/µl

Recommended Reaction Conditions

1X FastDigest™ Buffer
Incubation at 37°C

Methylation Effects on Digestion

Dam: never overlaps – no effect.

Dcm: may overlap – blocked.

CpG: never overlaps – no effect.

EcoKI: never overlaps – no effect.

EcoBI: may overlap – effect not determined.

Number of Recognition Sites in DNA

λ	Φ X174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
41	0	1	1	1	1	1

Thermal Inactivation

FastDigest™ AlwNI (Cail) is inactivated by incubation at 65°C for 10min.

Digestion of Plasmid DNA

1 μ l of FastDigest™ AlwNI (Cail) digests up to 1 μ g of plasmid DNA in 5min.

Digestion of PCR Products

1 μ l of FastDigest™ AlwNI (Cail) digests ~0.2 μ g of PCR product in 5min.

Digestion of Genomic DNA

1 μ l of FastDigest™ AlwNI (Cail) digests 1 μ g of genomic DNA in 5min, or 5 μ g of genomic DNA in 30min.

QUALITY CONTROL ASSAY DATA

Functional Activity Test

1 μ g of lambda DNA was completely digested with 1 μ l of the enzyme in 5 minutes at 37°C in 20 μ l of reaction mixture.

Ligation/Recutting Assay

After overdigestion with 1 μ l of FastDigest™ AlwNI (Cail) 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 occurred during incubation with 1 μ l of FastDigest™ AlwNI (Cail) for 1 hour.

Prolonged Incubation / Star Activity Assay

No detectable degradation of 1 μ g of lambda DNA due to nuclease contamination or star activity occurred during incubation with 1 μ l of FastDigest™ AlwNI (Cail) for 16 hours.

Quality authorized by:

 Jurgita Zilinskiene

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Protocol for Fast Digestion of DNA

① Combine the following reaction components at room temperature in the order indicated:

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

② Mix gently and spin down.

③ Incubate at 37°C in a heat block or water thermostat for 5min.

④ Inactivate the enzyme by heating for 10min 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 1µl 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

① Pipette 2µl of DNA* samples into tubes

② Prepare a master mix for n+1 samples

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

Water*, nuclease-free (#R0581)	$(10+1) \times 15\mu\text{l} = 165\mu\text{l}$
10X FastDigest™ buffer	$(10+1) \times 2\mu\text{l} = 22\mu\text{l}$
FastDigest™ enzyme	$(10+1) \times 1\mu\text{l} = 11\mu\text{l}$

③ Add 18µl of master mix* into tubes containing DNA.

* The volume of DNA can be scaled up to 10µl or down to 0.5µl 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	1µg	2µg	3µg	4µg	5µg
FastDigest™ enzyme	1µl	2µl	3µl	4µl	5µl
10X FastDigest™ buffer	2µl	2µl	3µl	4µl	5µl
Total volume:	20µl	20µl	30µl	40µl	50µl

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 3-5min if total reaction volume exceeds 20µl. 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.