Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

FastDigest® SanDI (KflI)*

#FD2164 20 µl (for 20 reactions)
Lot: **Expiry Date:**

5'...**G G↓G W C C C**...3'
3'...**C C C W G↑G G**...5'

* FastDigest® SanDI (KflI) is a proprietary formulation of KflI, an isoschizomer of SanDI having the same recognition and cleavage specificity.

Supplied with: 1 ml of 10X FastDigest® Buffer
1 ml of 10X FastDigest® Green Buffer

Store at -20°C

In total 3 vials. BSA included

ISO 9001 ISO 14001
www.fermentas.com

Description

FastDigest® enzymes are an advanced line of restriction enzymes for rapid DNA digestion in 5 - 15 minutes.
All FastDigest® enzymes are 100% active in the universal FastDigest® and FastDigest® Green buffers. Enzymes used in common downstream applications such as ligation, blunting and dephosphorylation reactions also have 100% activity in FastDigest® and FastDigest® Green Buffer.
The FastDigest® Green Buffer includes a density reagent along with blue and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The blue dye migrates with 3-5 kb DNA fragments in a 1% agarose gel and has an excitation peak at 424 nm. The yellow dye migrates faster than 10 bp DNA fragments in a 1% agarose gel and has an excitation peak at 615 nm. The presence of the dyes in the FastDigest® Green Buffer does not interfere with DNA digestion or with downstream applications. However, the dyes may interfere with some fluorescence measurements. We recommend using the colorless FastDigest® Buffer for applications that require analysis of the digestion product by fluorescence excitation.

Recommended Reaction Conditions

- 1X FastDigest® Buffer or 1X FastDigest® Green Buffer.
- Incubation at 37°C.
- 1 µl of FastDigest® SanDI (KfiI) is formulated to digest up to:
  – 1 µg of lambda DNA in 5 min.
  – 1 µg of plasmid DNA in 5 min.
  – 0.2 µg of PCR product in 5 min.
  – 1 µg of genomic DNA in 10 min, or 5 µg of genomic DNA in 60 min.

Inactivation

Phenol/chloroform extraction and ethanol precipitation of DNA. Thermal inactivation is not applicable for FastDigest® SanDI (KfiI).

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: may overlap – effect not determined.
CpG: may overlap – cleavage impaired.
EcoKI: never overlaps – no effect.
EcoBI: never overlaps – no effect.

Compatible Ends

FastDigest® AvaII (Eco47I), FastDigest® Eco0109I, FastDigest® PpuMI (Psp5II), FastDigest® RsrII (CpoI), FastDigest® Sau96I (Cfr13I), CpoI, Cfr3I, Eco47I, Eco0109I, Psp5II.

Number of Recognition Sites in DNA

Ad2	λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
8	1	0	0	0	0	0	0

QUALITY CONTROL ASSAY DATA

Functional Activity Test

1 µg of linearized pJET1 DNA with inserted SanDI (KfiI) recognition sites was completely digested with 1 µl 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® SanDI (KfiI) for 1 hour, more than 90% 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® SanDI (KfiI) for 1 hour.

Prolonged Incubation / Star Activity Assay

No detectable degradation of 1 µg of linearized pJET1 DNA with inserted SanDI (KfiI) recognition site due to nuclease contamination or star activity occurred during incubation with 1 µl of FastDigest® SanDI (KfiI) for 16 hours.

Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/SmaI and pUC57/PstI digests was incubated with 1 µl of FastDigest® SanDI (KfiI) for 16 hours. After religation and transformation, the background level of white colonies was <1%.

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® or 10X FastDigest® Green 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 (plasmid and PCR product), or for 10 min (genomic DNA).

❹ Inactivate the enzyme by phenol/chloroform extraction (optional).

❺ If the FastDigest® Green Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

Double and Multiple Digestion of DNA

- Use 1 µl of each enzyme and scale up the reaction conditions appropriately. If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.
- The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume.

Activity of DNA Modifying Enzymes in FastDigest® and FastDigest® Green Buffers, %

FastAP™ Thermosensitive Alkaline Phosphatase	100
Shrimp Alkaline Phosphatase	100
T4 DNA Ligase*	75-100
Klenow Fragment	100
T4 DNA Polymerase	100
T4 Polynucleotide Kinase	100

***** 0.5 mM ATP is required for T4 DNA Ligase activity.

Scaling up a 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® or 10X FastDigest® Green 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 the enzyme to DNA methylation (*see* **Methylation Effects on Digestion**).
- The context of the target sequence may affect DNA cleavage efficiency. A 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 "Reaction Conditions for FastDigest® Restriction Enzymes" (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 efficiency.
- Increase the incubation time by 3-5 min if the total reaction volume exceeds 20 µl. Air thermostats are not recommended due to the slow transfer of heat 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.