Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific FastDigest KflI*

#FD2164
20 µL (for 20 rxns)
Lot: _____ Expiry Date: _____

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

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

Supplied with: 1 mL of 10X FastDigest Buffer
1 mL of 10X FastDigest Green Buffer

Store at -20°C

Description

Thermo Scientific FastDigest enzymes are an advanced line of restriction enzymes for rapid DNA digestion. All FastDigest™ enzymes are 100% active in the universal FastDigest and FastDigest Green buffers and are able to digest DNA in 5-15 minutes. This enables any combination of restriction enzymes to work simultaneously in one reaction tube and eliminates the need for sequential digestions. FastDigest enzymes can be used to digest plasmid, genomic and viral DNA as well as PCR products and do not show star activity even in prolonged incubations.
Enzymes used in common downstream applications such as ligation, blunting and dephosphorylation reactions also have 100% activity in FastDigest and FastDigest Green Buffer.
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 of the FastDigest Green Buffer migrates with 3-5 kb DNA fragments in a 1% agarose gel and has an excitation peak at 424 nm.
The yellow dye of the FastDigest Green Buffer migrates faster than 10 bp DNA fragments in a 1% agarose gel and has an excitation peak at 615 nm.
For applications that require analysis by fluorescence excitation FastDigest Buffer is recommended, as the dyes of the FastDigest Green Buffer may interfere with some fluorescence measurements.

Recommended Reaction Conditions

- 1X FastDigest Buffer or 1X FastDigest Green Buffer.
- Incubation at 37°C.
- 1 µL of FastDigest KflI 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 KflI.

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

Check www.thermoscientific.com/research for the list of restriction enzymes producing compatible ends.

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

CERTIFICATE OF ANALYSIS

Functional Activity Test

1 µg of linearized pJET1 DNA with inserted KflI recognition sites was completely digested with 1 µL of the enzyme in 5 minutes at 37°C in 20 µL of reaction mixture.

Ligation and Recleavage (L/R) Assay

The ligation and recleavage assay was replaced with LO test after validating experiments showed LO test ability to trace nuclease and phosphatase activities with sensitivity that is higher than L/R by a factor of 100.

Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded oligonucleotides occured during incubation with 1 µL of FastDigest KflI for 1 hour.

Prolonged Incubation / Star Activity Assay

No detectable degradation of 1 µg of linearized pJET1 DNA with inserted KflI recognition sites due to nuclease contamination or star activity occurred during incubation with 1 µL of FastDigest KflI for 16 hours.

Blue/White (B/W) Cloning Assay

The B/W assay was replaced with LO test after validating experiments showed LO test ability to detect nuclease and phosphatase activities with sensitivity that equals to that of B/W test.

Quality authorized by:  Jurgita Zilinskiene

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Protocol for Fast Digestion of Different DNA

1. 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

2. Mix gently and spin down.
3. Incubate at 37°C in a heat block or water thermostat for 5 min (plasmid and PCR product), or for 10 min (genomic DNA).
   Optional: Inactivate the enzyme by phenol/chloroform extraction.
4. If the FastDigest Green Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

Note: The FastDigest Green Buffer can be used as an electrophoresis loading buffer for any DNA sample at a final 1X concentration. Higher concentrations of FastDigest Green Buffer in the sample supply excess salt concentration which may alter DNA mobility.

Double and Multiple Digestion of DNA

- The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total reaction volume.
- 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.

Scaling up Plasmid 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

Note: Increase the incubation time by 3-5 min if the total reaction volume exceeds 20 µL. Use water thermostat, air thermostats are not recommended due to the slow transfer of heat to the reaction mixture.

Recommendations for PCR product digestion

- When introducing restriction enzyme sites into primers for subsequent digestion and cloning of a PCR product, refer to www.thermoscientific.com/fd, Reaction Conditions Guide, to define the number of extra bases required for efficient cleavage.
- Use Thermo Scientific GeneJET PCR Purification Kit, #K0701 to purify PCR product prior digestion in following cases:
  – When PCR additives such as DMSO or glycerol where used, as they may affect the cleavage efficiency or cause star activity.
  – When PCR Product will be used for cloning. Active thermophilic DNA polymerase still present in PCR mixture may alter the ends of the cleaved DNA and reduce the ligation efficiency.

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

Thermo Scientific FastAP Thermosensitive Alkaline Phosphatase, #EF0651	100
T4 DNA Ligase*, #EL0014	75-100
Klenow Fragment, #EP0051	100
T4 DNA Polymerase, #EP0061	100
T4 Polynucleotide Kinase, #EK0031	100

* 0.5 mM ATP (#R0441) is required for T4 DNA Ligase activity.

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.thermoscientific.com/onebio for Material Safety Data Sheet of the product.