Thermo SCIENTIFIC

PRODUCT INFORMATION

Thermo Scientific FastDigest AjuI

#FD1954 20 µL (for 20 rxns)

Lot: _____ **Expiry Date:** _____

5'...↓ $_{7}$(N)G A A(N)$_{7}$ T T G G(N)$_{11}$↓...3'

3'...↑$_{12}$(N)C T T(N)$_{7}$ A A C C(N)$_{6}$ ↑...5'

Supplied with: 1 mL of 10X FastDigest Buffer
1 mL of 10X FastDigest Green Buffer
20 µL of 20X SAM (0.2 mM)

Store at -20°C

F SAM 37° 5' 65° LO

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 + 0.01 mM SAM.
- Incubation at 37°C.
- 1 µL of FastDigest AjuI is formulated to digest up to:
  - 1 µg of lambda DNA in 15 min.
  - 1 µg of plasmid DNA in 15 min.
  - 0.2 µg of PCR product in 10 min.
  - 1 µg of genomic DNA in 5 min, or 5 µg of genomic DNA in 30 min.

Thermal Inactivation: Incubation at 65°C for 5 min.

Methylation Effects on Digestion

Dam: never overlaps – no effect.
Dcm: never overlaps – no effect.
CpG: may overlap – no effect.
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

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
3	1	0	0	0	0	0

Note

- FastDigest AjuI requires S-adenosylmethionine for activity. Still, complete cleavage of some substrates by FastDigest AjuI is difficult to achieve.
- FastDigest AjuI produces double-strand cuts on both sides from the interrupted recognition site. The exact cleavage position depends on the sequences flanking the recognition site and may shift by one base pair. However, for each individual sequence one cleavage position will dominate.

CERTIFICATE OF ANALYSIS

Functional Activity Test

1 µg of ΦX174 DNA was completely digested with 1 µL of the enzyme in 15 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 AjuI for 1 hour.

Prolonged Incubation / Star Activity Assay

No detectable degradation of 1µg of ΦX174 DNA due to nuclease contamination or star activity occurred during incubation with 1 µL of FastDigest AjuI 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)	14 µL	15.5 µL	27.5 µL
10X FastDigest or 10X FastDigest Green Buffer	2 µL	2 µL	5 µL
20X SAM (0.2 mM)	1 µL	1.5 µL	2.5 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 genomic DNA), or for 10 min (PCR product). Optional: Inactivate the enzyme by heating for 5 min at 65°C.

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
20X SAM (0.2 mM)	1 µL	1 µL	1.5 µL	2 µL	2.5 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.