FastDigest Alw26I (IIs class)

Catalog Number FD0034

Pub. No. MAN0012416 **Rev.** B.00

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

5'...G T C T C (N)$_1$↓...3'
3'...C A G A G (N)$_5$↑...5'

Contents and storage

Cat No.	Contents	Amount	Storage
FD0034	FastDigest Alw26I (IIs class)	100 µL, 100 reactions	-25 °C to -15 °C
	10X FastDigest Buffer	1 mL	
	10X FastDigest Green Buffer	1 mL	

BSA included.

Description

FastDigest Alw26I (IIs class) belongs to specific class of restriction enzymes which recognize asymmetric DNA sequences and cleave outside of their recognition sequence. IIs class enzymes are useful for many applications, including their use in seamless cloning of multiple fragments in a predefined order (1, 2, 3).

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 are optimized 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 have100 % 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

Reaction temperature	Digestion time with 1 µL of FastDigest enzyme, min				bp from end of DNA required for complete digestion	Thermal inactivation	Incubation time without star activity, hours
	Lambda 1µg/20 µL	Plasmid DNA, 1 µg/20 µL	PCR product, ~0.2 µg/30 µL	Genomic DNA, 1 µg/10µL			
37 °C	5	5	5	5	1	65 °C, 5 min	16

Methylation effects on digestion

Methylation type	Sequence	Cleavage effect
CpG	5'…GTCTm5C G…3' 3'…CAGA Gm5C…5'	No effect
CpG	5'…m5C GTCTC…3' 3'… Gm5CAGAG…5'	Blocked

Number of recognition sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19
37	4	3	4	4	2	5

Protocol for fast digestion of different DNA

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

Component	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. *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

Component	1 µg DNA digestion	2 µg DNA digestion	3 µg DNA digestion	4 µg DNA digestion	5 µg DNA digestion
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.thermofisher.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, %

Enzymes	Cat #	Activity, %
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.

Reference

1. Engler, C., Gruetzner, R., Kandzia, R., and Marillonnet, S. (2009) Golden gate shuffling: a one-pot DNA shuffling method based on type IIs restriction enzymes. PLoS One 4, e5553.
2. Engler, C., Kandzia, R., and Marillonnet, S. (2008) A one pot, one step, precision cloning method with high throughput capability. PLoS One 3, e3647.
3. Engler, C., and Marillonnet, S. (2013) Combinatorial DNA assembly using Golden Gate cloning. Methods Mol Biol 1073, 141-156.

Limited product warranty

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