FastDigest restriction enzymes

Protocol for rapid digestion of DNA using Thermo Scientific™ FastDigest™ restriction enzymes

Prepare the reaction mixture at room temperature in the order indicated:

Component	Volume		
	Plasmid DNA	PCR product**	Genomic DNA
Water,* nuclease-free (Cat. No. R0581)	15 µL	16 µL	30 µL
10X FastDigest™ Green Buffer or 10X FastDigest™ Buffer	2 µL	3 µL	5 µL
DNA*	2 µL (up to 1 µg)	10 µL (~0.2 µg)	10 µL (up to 5 µg)
FastDigest enzyme	1 µL	1 µL	5 µL
Total volume	**20 µL**	**30 µL**	**50 µL**

1. Mix gently and spin down.
2. Incubate at 37°C in a heat block or water bath for 5 min.†
3. (Optional) Inactivate the enzyme.†
4. If FastDigest Green Buffer was used, load a portion of the reaction mixture directly onto a gel.

Scaling up DNA digestion reactions

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 Green Buffer or 10X FastDigest Buffer	2 µL	2 µL	3 µL	4 µL	5 µL
Total volume	**20 µL**	**20 µL**	**30 µL**	**40 µL**	**50 µL**

Digestion of DNA with multiple enzymes

FastDigest enzymes allow for simultaneous digestion of DNA with two or more enzymes in one reaction.

- Use 1 µL of each enzyme and scale up the reaction conditions appropriately.
- The combined volume of all added enzymes should not exceed one-tenth of the total reaction volume.

Reaction setup for digestion of multiple DNA samples

1. Pipette 2 µL of DNA* into each tube.
2. Prepare a master mix for n + 1 samples.
3. Example of master mix for 10 samples:

Water,* nuclease-free (Cat. No. R0581)	(10 + 1) x 15 µL = 165 µL
10X FastDigest Green Buffer or 10X FastDigest Buffer	(10 + 1) x 2 µL = 22 µL
FastDigest enzyme	(10 + 1) x 1 µL = 11 µL

4. Add 18 µL of master mix* to tubes containing DNA.

* The volume of DNA can be adjusted depending on the DNA concentration. The volume of water and master mix should be adjusted to keep the indicated total reaction volume.

** Water and buffer volumes are for unpurified PCR products.

† See the product information sheet for enzyme- and substrate-specific incubation times and enzyme inactivation conditions.

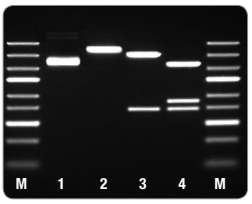
thermo scientific

Important considerations

- When planning experiments, make sure to check the sensitivity of a FastDigest enzyme to DNA methylation. Relevant information can be found on our website or in the product insert.
- The DNA sequence surrounding the recognition site may affect cleavage efficiency. Extend the incubation time to achieve complete digestion.
- Use the Thermo Scientific™ GeneJET™ PCR Purification Kit (Cat. No. K0701/2) for routine PCR reaction cleanup prior to digestion in the following cases:
  - PCR additives such as DMSO or glycerol were used. They may affect cleavage efficiency or cause star activity.
  - The PCR product will be used for cloning. Active thermophilic DNA polymerase still present in the PCR mixture may alter the ends of the cleaved DNA and reduce the efficiency of subsequent ligation reactions.
- Increase the incubation time by 3–5 min if the total reaction volume exceeds 20 µL. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.

Note: If the DNA needs to be concentrated into a small volume, we recommend using the Thermo Scientific™ GeneJET™ Gel Extraction and DNA Cleanup Micro Kit (Cat. No. K0831/2).

5 min digestions in FastDigest Green Buffer



M – Thermo Scientific™ GeneRuler™ Express DNA Ladder (Cat. No. SM1553)
1 – Undigested plasmid DNA
2 – Plasmid digested with FastDigest EcoRI
3 – Plasmid double-digested with FastDigest EcoRI and KpnI
4 – Plasmid triple-digested with FastDigest EcoRI, KpnI, and SmaI

Ordering information

DNA-modifying enzymes	Activity (%) in FastDigest or FastDigest Green buffer	Cat. No.
DNA Polymerase I	100	EP0041
Klenow Fragment	100	EP0051
Klenow Fragment, exo⁻	100	EP0421
T4 DNA Polymerase	100	EP0061
T7 DNA Polymerase	100	EP0081
T4 DNA Ligase*	75–100	EL0011
FastAP Thermosensitive Alkaline Phosphatase	100	EF0651
T4 Polynucleotide Kinase	100	EK0031

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

Find out more at **thermofisher.com/fastdigest**

ThermoFisher SCIENTIFIC