FastDigest™ Restriction Enzymes

Protocol for Fast Digestion of DNA

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

Component	Volume		
	Plasmid DNA	PCR product	Genomic DNA
Water*, nuclease-free (#R0581)	15 µl	17 µl	30 µl
10X FastDigest™ 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 5min**
❹ Inactivate the enzyme (optional)**

Double and Multiple Digestion of DNA

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

- Use 1µl of each enzyme and scale up the reaction conditions appropriately
- The combined volume of all added enzymes should not exceed 1/10 of the total reaction volume

Reaction Set-up for Digestion of Multiple DNA Samples

❶ Pipette 2 µl of DNA* samples into tubes
❷ Prepare a master mix for n+1 samples

Example of master mix (for 10 samples of plasmid DNA):

Water*, nuclease-free (#R0581)	(10+1) x 15 µl = 165 µl
10X FastDigest™ buffer	(10+1) x 2 µl = 22 µl
FastDigest™ enzyme	(10+1) x 1 µl = 11 µl

❸ Add 18µl of master mix* into tubes containing DNA

Scaling up 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™ buffer	2 µl	2 µl	3 µl	4 µl	5 µl
Total volume	20 µl	20 µl	30 µl	40 µl	50 µl

* *The volume of DNA can be scaled up to 10µl or down to 0.5µl depending on the DNA concentration. The volume of water and master mix should be corrected to keep the indicated total reaction volume.*
** *See the Certificate of Analysis for enzyme and substrate specific incubation time and enzyme inactivation conditions.*

FastDigest™ Restriction Enzymes

Activity of DNA/RNA Modifying Enzymes in FastDigest™ Buffer

DNA/RNA modifying enzyme	Activity in FastDigest™ buffer, %
DNA Polymerase I, *E.coli*	100
Klenow Fragment	100
Klenow Fragment, exo⁻	100
T4 DNA Polymerase	100
T7 DNA Polymerase	100
T4 DNA Ligase*	75-100
Bacterial Alkaline Phosphatase	100
Shrimp Alkaline Phosphatase	100
Calf Intestine Alkaline Phosphatase	100
T4 Polynucleotide Kinase	100

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

Visit **www.fermentas.com/fastdigest** for complete information and updated list of FastDigest™ enzymes.

Important Notes

- Always check the sensitivity of the enzyme to DNA methylation in the Certificate of Analysis or Catalog.
- The context of the target sequence may affect DNA cleavage efficiency. Prolong incubations to achieve complete digestion.
- PCR additives such as DMSO or glycerol may affect 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 "Cleavage efficiency close to the termini of PCR fragments" (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 from the PCR mixture. DNA polymerases may alter the ends of the cleaved DNA and reduce the ligation yield.
- Increase the incubation time by 3-5 min if total reaction volume exceeds 20 µl. Air thermostats are not recommended due to slow heat transfer to the reaction mixture.