

FlashDigest NcoI

Troubleshooting Guide

30 rxn

Cat No: FD0009

Use this guide for common digestion, handling, and downstream-workflow problems encountered with FlashDigest NcoI. FlashDigest enzymes are designed for rapid digestion of plasmid DNA, PCR products, and genomic DNA in 10x FlashDigest Buffer or 10x FlashDigest Red Buffer.

Before You Start - Quick Checks

1. Store the enzyme at -20°C and prepare restriction reactions on ice.
2. For plasmid DNA: up to 1 µg DNA, 2 µL 10x buffer, 1 µL enzyme, 20 µL total volume.
3. For PCR products: up to ~0.2 µg DNA, 3 µL 10x buffer, 1 µL enzyme, 30 µL total volume; unpurified PCR products may require special handling as noted in the datasheet.
4. For genomic DNA: 5 µg DNA, 5 µL 10x buffer, 5 µL enzyme, 50 µL total volume.
5. Mix gently by pipetting or tapping; do not vortex. Briefly centrifuge to collect droplets.
6. Incubate at 37°C for 15 min (plasmid), 15-30 min (PCR product), or 30-60 min (genomic DNA); optional inactivation is 80°C for 20 min.

Problem	Most likely cause	Recommended action
Incomplete or partial digestion	DNA input, enzyme amount, buffer volume, or incubation time may not match the recommended setup.	For plasmid DNA use up to 1 µg with 1 µL enzyme in 20 µL; for PCR product up to ~0.2 µg with 1 µL enzyme in 30 µL; for genomic DNA use 5 µg with 5 µL enzyme in 50 µL. Incubate at 37°C for the recommended time.
PCR product digests poorly	Unpurified PCR products may contain residual salts or enzymes that reduce digestion efficiency.	Purify PCR products before digestion for optimal downstream results. If using an unpurified PCR product directly, follow the datasheet note regarding buffer volume.
Genomic DNA digestion is incomplete	Genomic DNA requires a longer incubation and more enzyme than plasmid DNA or PCR products.	Use the genomic DNA setup in the datasheet and incubate at 37°C for 30-60 minutes.
No expected restriction fragments are observed	The DNA may not contain the expected NcoI cleavage site or the predicted restriction pattern may be incorrect.	Confirm the target sequence and expected NcoI cleavage sites before repeating the reaction. Use the substrate cleavage-site information in the datasheet as a reference where applicable.
Double or multi-enzyme digestion is inefficient	The combined enzyme volume may be too high or reaction proportions may not have been adjusted correctly.	Use 1 µL of each FlashDigest endonuclease per reaction and keep the combined rapid-endonuclease volume at no more than 10% of the total reaction volume.
Unexpected nonspecific or star-like pattern	The reaction may have been incubated much longer than required.	Use the recommended digestion time. The datasheet notes that extended incubation may increase the risk of star activity.
Red Buffer tracking dyes are confused with DNA bands	The red and yellow tracking dyes migrate independently of the DNA fragments.	In a 1% agarose gel, use the red dye (~2500 bp migration) and yellow dye (~10 bp migration) only as tracking references, not as DNA bands.
Downstream cloning or ligation is poor	PCR-product contaminants, incomplete digestion, or an inappropriate downstream cleanup step may be affecting the result.	For cloning workflows, purify PCR products before digestion as recommended, confirm complete digestion, and purify digestion products when required by the downstream protocol.
Residual restriction activity affects the next step	The optional heat-inactivation step may have been omitted when inactivation was needed.	If the downstream workflow requires enzyme inactivation, incubate at 80°C for 20 minutes as specified, or use the appropriate cleanup method for the application.

Still having trouble? Confirm the DNA input, 10x buffer volume, FlashDigest NcoI amount, and 37°C incubation time for the sample type. For PCR products, consider purification before digestion. For double/multi-digests, keep the combined enzyme volume within 10% of the total reaction volume and avoid unnecessarily prolonged incubation.