

FlashDigest DpnI

Troubleshooting Guide

50 rxn

Cat No: FD0011

Use this guide for common digestion, handling, and downstream-workflow problems encountered with FlashDigest DpnI. DpnI is methylation-dependent: it cleaves methylated GATC sites and is commonly used after PCR to remove methylated parental plasmid template.

Before You Start - Quick Checks

1. Store FlashDigest DpnI at -20°C and prepare restriction reactions on ice.
2. DpnI specifically cleaves methylated GATC sites; standard unmethylated PCR amplicons are not expected to be digested.
3. Plasmid DNA: up to 1 µg DNA, 2 µL 10x buffer, 1 µL DpnI, 20 µL total. PCR product: up to ~0.2 µg, 3 µL buffer, 1 µL DpnI, 30 µL total.
4. Genomic DNA: 5 µg DNA, 5 µL 10x buffer, 5 µL DpnI, 50 µL total.
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: 80°C for 20 min.

Problem	Most likely cause	Recommended action
Standard PCR amplicon is not cut or remains intact	DpnI specifically cleaves methylated GATC sites. Standard PCR amplicons are generally unmethylated and therefore are not expected to be digested.	This is expected behavior for an unmethylated PCR product. In post-PCR workflows, use DpnI to selectively remove the methylated parental plasmid template. PCR cleanup does not create DNA methylation.
Parental plasmid persists after post-PCR DpnI treatment	The parental template may be insufficiently exposed to enzyme because of reaction composition, DNA amount, or inhibitory carryover from the PCR mixture.	Confirm that the parental plasmid template is methylated. Use the recommended PCR-product reaction setup and incubate at 37°C for 15-30 min. If direct digestion is inefficient, purify the PCR product before digestion.
Incomplete digestion of plasmid DNA	DNA input, enzyme amount, buffer volume, or incubation conditions do not match the recommended plasmid setup.	Use up to 1 µg plasmid DNA with 2 µL 10x FlashDigest Buffer or Red Buffer and 1 µL DpnI in a 20 µL reaction. Incubate at 37°C for 15 min.
Digestion is poor in an unpurified PCR reaction	Residual salts or enzymes in unpurified PCR products may reduce digestion efficiency.	For optimal downstream results, purify PCR products before digestion. If using an unpurified PCR product directly, the datasheet allows the 10x buffer volume to be reduced to 2 µL.
Genomic DNA digestion is incomplete	Genomic DNA requires more enzyme and a longer incubation than plasmid DNA.	Use 5 µg genomic DNA, 5 µL 10x buffer, and 5 µL DpnI in a 50 µL reaction. Incubate at 37°C for 30-60 min.
No expected restriction pattern is observed	The DNA may lack a DpnI cleavage site, or the GATC site may be unmethylated.	Confirm both the expected DpnI site pattern and methylation status of the substrate. Sequence presence alone is not sufficient for cleavage by DpnI.
Unexpected nonspecific or star-like pattern	The digestion 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.
Double or multi-enzyme digestion is inefficient	The combined enzyme volume may be too high or the reaction proportions may not have been scaled correctly.	Use 1 µL of each FlashDigest endonuclease per reaction and keep the combined enzyme volume at no more than 10% of the total reaction volume.
Red Buffer tracking dyes are confused with DNA bands	The tracking dyes migrate independently of DNA fragments.	In a 1% agarose gel, the red dye migrates similarly to ~2500 bp DNA and the yellow dye to ~10 bp DNA. Treat them only as tracking references.
Downstream cloning or ligation is poor	PCR contaminants, incomplete digestion, or residual reaction components may interfere with downstream steps.	Purify PCR products before digestion when cloning is planned, verify digestion, and clean up digestion products when required by the downstream workflow.
Residual DpnI activity affects the next step	The optional heat-inactivation step was omitted when enzyme inactivation was needed.	Incubate at 80°C for 20 min to inactivate DpnI when appropriate, or use a suitable cleanup method before the next application.

Still having trouble? First confirm that the substrate contains methylated GATC sites. Then verify DNA input, buffer volume, DpnI amount, and the 37°C incubation time for the sample type. For post-PCR workflows, remember that DpnI is intended to remove methylated parental plasmid DNA; standard unmethylated PCR amplicons are expected to remain intact.