Dpn I

Product description

Dpn I is a Dam methylation-sensitive restriction enzyme that recognizes GA/TC sequences, and can only cleave DNA when adenine N6-methylation is present in the sequence. The digestion will be blocked when CpG methylation of mammalian DNA overlaps with restriction sites of Dpn I. In addition, Dpn I had no effect on Dcm methylation. This product is suitable for rapid digestion reactions of plasmid DNA, PCR products or genomic DNA, and is widely used in many research fields such as genotyping, molecular cloning, Southern blotting, SNP, RFLP technology, etc.

The restriction sites of Dpn I as follows:

5'…… G $A^{m6}\downarrow$T C…… 3'

3'…… C $T\uparrow$ A^{m6}G…… 5'

Components and storage conditions

Components \ Size	500 U	1000 U	5000 U
Dpn I (20 U/μL)	0.025 mL	0.05 mL	0.25 mL
10X Dpn I Reaction Buffer	0.5 mL	1 mL	5 mL
Store the components at -20 °C.			

Experimental operation

DNA digestion:

1. Configure the reaction system on ice according to the following table:

Total Reaction Volume	50 μL	
DNA	X μL	1 μg
10X Dpn I Reaction Buffer	5 μL	
Dpn I	0.5~1 μL	
Nuclease-free Water	X μL	To 50 μL

2. Mix the reaction system Gently, then centrifuge rapidly to collect the residual liquid from the tube wall.
3. Incubate at 37 °C for 1 h.

4. Heating at 80 °C for 20 min to inactivate Dpn I (optional).

Notes

1. Dpn I should be added last when configuring the reaction system. Keep Dpn I on ice when removing it from the freezer.
2. Mix the components by repeat pipetting or "flicking" the tube. Do not mix the reactants by vortexing.
3. If it is found that the intended digestion site cannot be cleaved, confirm whether the site has been methylated. Note that Dpn I will only cleave fully-adenomethylated dam sites. Hemi-adenomethylated dam sites slowed the digestion of Dpn I by about 60 times.
4. For double or multi-enzyme digestion, select an appropriate buffer that is compatible with two or more endonucleases and refer to the table above to set up the reaction system. If a suitable buffer is not available, the product can be purified and collected after digestion with one enzyme, followed by another digestion reaction.

Product characteristics

1. Enzyme activity unit (U) definition: The amount of enzyme required to digest 1 μg of pBR322 DNA (Dam methylation) at 37°C for 1 h in a 50 μL reaction containing Dpn I Reaction Buffer.
2. Stored solution composition: 10 mM Tris-HCl(pH 7.4), 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 200 μg/mL Recombinant Albumin, 50% Glycerol.
3. 10X Dpn I Reaction buffer: 200 mM Tris-acetate(pH 7.9 @ 25°C), 500 mM Potassium Acetate, 100 mM Magnesium Acetate, 1 mg/mL Recombinant Albumin.
4. Quality assurance:
   - Purity: SDS-PAGE detection purity > 95%.
   - No non-specific nuclease, endonuclease or exonuclease.
   - No RNase.
5. Inactivation conditions: Dpn I can be inactivated by heating at 80 °C for 20 min.
6. This product is for scientific use only.

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