Template Eliminator

REF: EG21203S

Storage Condition

-20℃

Components

Component	Amount
Template Eliminator	50 μl

Description

Template Eliminator is an enzyme developed based on DpnI and SgeI with improved plasmid digestion efficiency. This enzyme is obtained by mixing DpnI and SgeI in certain proportions. Since DpnI cleaves Dam^+ substrates and SgeI cleaves Dcm^+ substrates, the combination of the two can complement each other, thereby enhancing the efficiency of plasmid digestion.

DpnI requires the presence of N6-methyladenine in the recognition sequence to cleave DNA. DNA purified from Dam^+ strains will serve as the substrate for DpnI. DpnI can only cleave fully adenine-methylated Dam sites, with a 60-fold slower cleavage rate for hemi-methylated Dam sites. SgeI can cleave DNA targets containing 5-methylcytosine on single-stranded or double-stranded DNA. DNA methylated by Dcm or CpG methyltransferases will serve as the substrate for SgeI. Effective cleavage requires at least two SgeI recognition sequences to be replicated. The amount of enzyme required for complete cleavage of methylated DNA depends on the number of SgeI recognition sites. Therefore, mixing DpnI and SgeI in certain proportions can cover more usage scenarios, accommodate more host types, and increase the flexibility of point mutation.

It has been verified that this product has a plasmid residual rate of only 10~20% of DpnI and only 0.1~1% of SgeI for different types of templates. Additionally, this enzyme can adapt to conventional PCR reaction buffers, allowing for direct digestion after amplification.

Quality Control Assays

Function

Using 1 μl Template Eliminator to digest the pUC19 point mutant product, the blue-white spot screening showed that the proportion of background spots after digestion was <2%.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid (Dam^-, Dcm^-) and 1 μl of Template Eliminator incubated for 4 hours at 37 ℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 1 μl of Template Eliminator incubated for 1 hour at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount
PCR product[b]	X[a] μl
Template Eliminator	1 μl
Nuclease-Free Water	To 20 μl

a. The amount of PCR product can be flexibly selected according to the amount of amplified product. If the amount of product is large, the amount of use can be reduced, and if the amount of product is small, the amount of use can be increased.

b. For regular PCR buffers, this product can be well adapted. For some PCR buffers with high salt ion concentration, it is recommended to reduce the amount of PCR products during the reaction, generally not exceeding 1/2 of the reaction volume.

2. Vortex and mix, then centrifuge briefly.

3. Incubate at 37℃ for 15 minutes.

4. After the reaction is completed, it can be directly used for transformation, or stored at -20℃ for future use.

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