Product Specification

Product Name:	SpeedyCut *KpnI* Restriction Enzyme
Product Code:	SGRE02200

Product Description:

KpnI is categorized as a SpeedyCut series Restriction Enzymes (RE). It is a subset of RE that are capable of cutting DNA within 5 – 15 minutes. All SpeedyCut RE are compatible with the universal SpeedyOne buffer, this universal buffer allows for combination of different enzymes within a standardized reaction system without the need for multiple rounds of single enzyme cuts. They are convenient, stable, fast and can accurately cut DNA.

KpnI is a type of Type II class RE. The SpeedyCut series RE undergoes strict quality control processes to ensure its reliability for rapid digestion of plasmid DNA, genomic DNA and PCR products.

***KpnI* Recognition Site**
5'…-GGTAC↓C-…3' 3'…-C↑CATGG-…5

Product Content(s)

SpeedyCut *KpnI* RE	200µl
10X SpeedyOne buffer	1ml

Storage: -20ºC

Note: At 37ºC, in a 20µl reaction system, 1µl enzyme can completely digest 1µg λDNA in 15 minutes. If there's a difference in the optimal temperatures of the chosen enzymes, consider starting the digestion reaction with the enzyme having the lower optimal temperature, and then subsequently add the enzyme with the higher optimal temperature.

Procedures:

Rapid DNA digestion process

Plasmid DNA

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	2µl
DNA (1µg/µl)	2µl
SpeedyCut *KpnI*	1µl
Nuclease – free water	15µl
Total	20µl

Note: The volume of all enzyme added should not exceed 10% of the total volume to prevent an excess of glycerol in the enzyme, which may lead to star activity.

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PCR Product

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	3µl
DNA (0.2µg)	10µl
SpeedyCut *KpnI*	1µl
Nuclease – free water	16µl
Total	30µl

Genomic DNA

a. Begin by preparing the reaction setup, ensuring that all required reagents are kept on ice

Reagents	Volume
10X SpeedyOne Buffer	5µl
DNA (5µg)	10µl
SpeedyCut *KpnI*	5µl
Nuclease – free water	30µl
Total	50µl

Note: The above three systems are suitable for enzymatic digestion of purified PCR products. In case of unpurified PCR products, it contains a certain ion strength, the 10X SpeedyOne buffer added can be reduced to 2µl. However, due to the concurrent exonuclease activity of DNA polymerase, it is highly advised to purify the PCR products before proceeding with enzymatic digestion

b. Pipette all reagents into a microcentrifuge tube (according to the tables above) and gently resuspend to ensure it is homogenized (Do not Vortex). Immediately centrifuge the tubes to ensure no reagents are left on the side of the tube.
c. Incubate at 37°C for 15 minutes for plasmid DNA ,15 – 30 minutes for PCR products or 30 – 60 minutes for genomic DNA.
d. Optional, purification method like spin column or phenol / chloroform extraction could be used to stop the reaction.

Enzyme properties

1. Methylation Effects

a. Dam: Never overlap – no effects
b. Dcm: Never overlap – no effects
c. CpG: Never overlap – no effects
d. *EcoKI*: Never overlap – no effects
e. *EcoBI*: Never overlap – no effects

2. Heat Inactivation

a. Enzyme cannot be heat inactivated, use purification method like spin column or phenol / chloroform extraction

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3. Number of recognition sites in DNA

a. λDNA – 2
b. φX174 - 0
c. M13mp18/19 - 1
d. pBR322 – 0
e. pUC57 – 7
f. pUC18/19 – 1
g. SV40 - 1
h. Adeno2 - 8

Isochizomers

a. Asp7181, Acc65I

* Isochiomers may have different sensitivity to different methylation modification

Note:
This product is designed for research use only. Not to be used on humans or animals' diagnosis and other in vivo experiments.

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