SuperCut Fast Restriction Enzymes XhoI

Cat#: 8052441

[Product Name]

SuperCut Fast Restriction Enzymes XhoI

[Model & Size]

Product Name	Cat No.	Size	Component No.	Component Name	Component Size	Quantity
SuperCut Fast Restriction Enzymes XhoI	8052441	5000 units	8052441-1	SuperCut Fast Restriction Enzymes XhoI (10 units/μL)	5000 units	1 bottle
			8052441-2	SuperCut 10×Buffer	1 mL	3 bottle
			8052441-3	6× Gel Loading Buffer	1 mL	1 bottle

[Product Description]

SuperCut Fast Restriction Enzymes are a series of genetically engineered restriction enzymes that can accurately complete DNA digestion in 5~15 minutes, suitable for rapid digestion of plasmid DNA, PCR products or genomic DNA.

SuperCut Fast Restriction Enzymes has the following characteristics: digestion can be completed within 5~15 minutes; share a digestion buffer, which greatly simplifies the digestion reaction system; good enzyme activity redundancy for easy substrate overload or complex template digestion. In addition, dephosphorylation and ligation reagent of Biosci is 100% active in SuperCut Buffer, supporting one-tube reaction and improving the experience of "digestion-ligation-redigestion".

[Restriction Enzyme Site]

5'…C↓T C G A G…3'
3'…G A G C T↑C…5'
Isoschizomers*:PaeR7I, TliI, BssHI, Sfr274I, SlaI, StrI
***Isoschizomers may have different methylation sensitivities.**

[Storage And Transportation]

Store at -25℃~-15℃ for 24 months.
Transported on blue ice.

[Activity Definition]

1 unit is defined as the amount of enzyme required to digest 1 μg of λDNA in 50 μL the reaction at the optimal reaction temperature in 60 minutes.

[Recommended Reaction Conditions]

1× SuperCut Buffer;
Incubate at 37℃;
Refer to "Protocol for Fast DNA Digestion" for reaction setup.

[Heat Inactivation]

1. Incubation at 80℃ for 20 minutes;

2. Add an appropriate amount of 6×Gel Loading Buffer according to the reaction system to terminate the reaction.

[Quality Control]

Functional Test

A 20 μL reaction in SuperCut Buffer containing 1 μg of λDNA (HindIII digest) and 10 units of SuperCut Fast Restriction Enzymes XhoI incubated for 15 minutes at 37℃ results in complete digestion as determined by agarose gel electrophoresis.

Prolonged Incubation / Star Activity Assay

A 20 μL reaction in SuperCut Buffer containing 1 μg of λDNA (HindIII digest) and 10 units of SuperCut Fast Restriction Enzymes XhoI incubated for 3 hours at 37℃ results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. Longer incubation may result in star activity.

Enzyme Digestion-Ligation-Redigestion Test

At the optimal reaction temperature, the DNA was digested using 10 units of SuperCut Fast Restriction Enzymes XhoI, and then the digestion product was recovered. The DNA fragments can be religated using an appropriate amount of T4 DNA Ligase at 22℃. After the ligation product is recovered again, the ligation product can be recut using SuperCutXhoI.

Non-specific Endonuclease Activity

At the optimal reaction temperature, 10 units of SuperCut Fast Restriction Enzymes XhoI was incubated with a 20 μL reaction in SuperCut Buffer containing 1 μg of supercoiled plasmid DNA for 4 h, and the plasmid DNA was still supercoiled detected using agarose gel electrophoresis.

Blue/White Screening Assay

An appropriate vector containing *lacZα* gene is digested by 10 units of SuperCut Fast Restriction Enzymes XhoI. The digested product is ligated and transformed into *E.coli* competent cell. On Luria-Bertani culture plate with X-Gal, IPTG and appropriate antibiotic, the successfully ligated β-galactosidase gene can be expressed and gives rise to a blue colony, while an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. SuperCut restriction enzymes must produce fewer than 1% white colonies.

[Operating Instruction]

1. Protocol for Fast DNA Digestion

① Combine the following reaction components on ice in the order indicated:

	Plasmid DNA	PCR product	Genomic DNA
DNA[a]	≤ 1 μg	≤0.2 μg	≤ 5 μg
SuperCut Fast Restriction Enzymes XhoI	10 units	10 units	30~50 units
SuperCut 10×Buffer	2 μL	3 μL	5 μL
ddH_2O	To 20 μL	To 30 μL	To 50 μL

a. DNA should be free of phenol, chloroform, ethanol, EDTA, detergents or high concentrations of salts, otherwise enzyme activity will be affected; Methylated DNA inhibits certain restriction enzyme digestion reactions.

② Gently suck or flick the tube wall to mix well (never vortex), then centrifuge instantaneously to collect reaction solution;

③ Incubate at 37℃ for 15 minutes (plasmid DNA) or for 15~30 minutes (PCR product) or for 30~60 minutes (genomic DNA);

④ Optional: Inactivate the enzyme by heating for 20 minutes at 80℃ or add an appropriate amount of 6× Gel Loading Buffer according to the reaction system to terminate the reaction.

2. Double and Multiple Digestion of DNA

① Use 10 units of each enzyme and scale up the reaction conditions appropriately;

② The combined volume of the enzymes in the reaction mixture should not exceed 1/10 of the total

reaction volume;

③ If the enzymes require different reaction temperatures, start with the enzyme that requires a lower temperature, then add the second enzyme and incubate at the higher temperature.

Note: If the total volume of reaction solution is larger than 20 μL, the incubation time should be increased appropriately, and water bath, metal bath, or sand bath should be used as much as possible.

[Number Of Recognition Sites In DNA]

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
1	1	0	0	0	0	0	6

[Methylation Effects On Digestion]

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	impaired	No effect	No effect

[Activity In Different Buffers]

	SuperCut Buffer	Thermo Scientific FastDigest Buffer	NEB CutSmart® Buffer	Takara QuickCut™ Buffer
Activity	100%	100%	100%	100%

Note: The activity data is from the standard reation test of Biosci Restriction Enzyme described above.

[Icon Descriptions]

This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.

The enzyme's optimum reaction temperature is 37℃.

Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.

The enzyme can be heat inactivated at by incubation 80℃ for 20 minutes.

3 hours incubation do not show star activity, but longer incubation may result in star activity.

[Description Of Product Symbol]

Product Symbol	Description	Product Symbol	Description
REF	Catalog Number	LOT	Batch Code
	Date of Manufacture		Manufacturer
	Keep away from light		Temperature limit
	Consult instructions for use		Use-by date

[Company Information]

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