I-SceI

REF: EG25504S

Storage Condition

-20℃

Components

Component	Amount
I-SceI (5 U/μl)	50 μl
pUC-I-SceI (100 ng/μl)	20 μl
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

Description

I-SceI is an intron-encoded endonuclease derived from a group II intron of *Saccharomyces cerevisiae*. It specifically recognizes and cleaves an 18 bp non-palindromic sequence (5'-TAGGGATAACAGGGTAAT-3'), forming a 3' overhanging sticky end with a length of 4 bp.

Definition of Activity Unit

One unit of activity refers to the amount of enzyme required to completely digest 1 μg of λDNA at 37℃ for 1 hour.

Recommended Reaction Conditions

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

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Quality Control Assays

Function

A 20 μl reaction in CutOne® Buffer containing 1 μl of I-SceI can completely digest 1 μg of pUC-I-SceI within 15 minutes at 37℃ .

Prolonged Incubation / Star Activity Assay

A 20 μl reaction in CutOne® Buffer containing 1 μg of pUC-I-SceI and 1 μl of I-SceI incubated for 3 hours at 37℃ results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Ligation and Recuting

After 10-fold over-digestion with I-SceI at 37 ℃ , >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃ . Of these ligated fragments, >95% can be recut with I-SceI as determined by agarose gel electrophoresis.

Icon Descriptions

37 The enzyme's optimum reaction temperature is 37℃ .

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

Notice

1.Homing enzymes do not have stringently defined recognition sequences. They can tolerate minor sequence changes, which only partially affect the cleavage reaction.

The precise region of the essential bases in the recognition sequence remains uncertain. The recognition sequence provided is a site that is recognized and cleaved by the enzyme.

2.Plasmid DNA was supplied at a concentration of 100 ng/μl. Digestion with I-SceI results in the production of a 2711 bp fragment. pUC-I-SceI confers ampicillin resistance. This plasmid can be transformed independently and then extracted in large quantities for use in other experiments.

BestEnzymes Biotech Co., Ltd.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com

Method of application

1. Protocol for Fast DNA Digestion

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

	Plasmid DNA	PCR product	Genomic DNA
ddH_2O	15 μl	16 μl	30 μl
10× CutOne® Buffer or 10× CutOne® Color Buffer	2 μl	3 μl[a]	5 μl
DNA	2 μl (up to 1 μg)	10 μl (~0.2 μg)	10 μl (5 μg)
I-SceI (5 U/μl)	1 μl	1 μl	5 μl
Total	20 μl	30 μl	50 μl

a. For purified PCR products. If the PCR products are not purified, amount of 10× CutOne® Buffer should be reduced to 2 μl due to the remaining metal ions in the unpurified PCR products. We recommend to purify PCR products before digestion if it will be used for cloning, because the exonuclease activity of some DNA polymerases may alter the end of cleaved DNA.

② Mix gently and spin down;
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℃ ;
⑤ If the CutOne® Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

2. Scaling up Plasmid DNA Digestion Reaction

DNA	1 μg	2 μg	3 μg	4 μg	5 μg
I-SceI (5 U/μl)	1 μl	2 μl	3 μl	4 μl	5 μl
10× CutOne® Buffer or 10× CutOne® Color Buffer	2 μl	2 μl	3 μl	4 μl	5 μl
Total	20 μl	20 μl	30 μl	40 μl	50 μl

Note: Increase the incubation time if the total reaction volume exceeds 20 μl.

Number of Recognition Sites in DNA

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

Methylation Effects on Digestion

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

Activity of DNA Modifying Enzymes in CutOne® and CutOne® Color Buffers

EG15208S Alkaline Phosphatase (Fast)	100%
EG15205S T4 DNA Ligase (Fast)*	100%

*ATP is required for T4 DNA Ligase activity.

Add: No.17 Huaguoshan Avenue,Lianyungang City, China
Tel: +86 (518) 8558 6628 • E-mail:support@best-enzymes.com • www.best-enzymes.com