I-CeuI

REF: EG25529S

37 20' 80

Storage Condition

Store at -20℃ for 2 years.

Components

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

Description

I-CeuI is a homing endonuclease derived from *Chlamydomonas eugametos*, obtained by recombinant expression and purification in Escherichia coli. I-CeuI specifically recognizes and cleaves a 27-bp non-palindromic sequence: 5'-TAACTATAACGGTCCTAAGGTAGCGAA-3', generating a 4-bp 3' overhang. Compared with I-SceI, I-CeuI has a longer recognition sequence, so its natural occurrence frequency in the genome is much lower (approximately once per 5.6×10^{17} random bases), resulting in higher specificity for gene editing applications.

Definition of Activity Unit

One unit of activity refers to the amount of enzyme required to completely digest 1 μg of pUC-HE 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-CeuI can completely digest 1 μg of pUC-HE within 15 minutes at 37℃ .

Prolonged Incubation / Star Activity Assay

A 20 μl reaction in CutOne® Buffer containing 1 μg of pUC-HE and 1 μl of I-CeuI 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-CeuI 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-CeuI as determined by agarose gel electrophoresis.

Icon Descriptions

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

20' 80 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-CeuI results in the production of a 2711 bp fragment. pUC-HE confers ampicillin resistance. This plasmid can be transformed independently and then extracted in large quantities for use in other experiments.

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Protocol

1. Protocol for DNA Digestion

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

	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-CeuI (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-CeuI (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 in Different Buffers*

	CutOne® Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	100%	100%	100%	100%

*The activity data come from the functional test described above.

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.

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