SgrAI

REF: EG25513S

Storage Condition

-20℃

Components

Component	Amount
SgrAI (10 U/μl)	50 μl
10× Cut Buffer B	1 ml

Description

SgrAI is derived from *Streptomyces griseus* and is obtained through purification following recombinant expression in Escherichia coli. It specifically recognizes and cleaves the CR/CCGGYG sequence. SgrAI belongs to the non-LightNing® restriction enzymes; however, it is compatible with CutOne® buffer, allowing for double digestion with other LightNing® restriction enzymes. Due to its specificity, SgrAI requires two or more restriction sites to achieve efficient cleavage, with significantly reduced cleavage efficiency for substrates containing a single site.

Recommended Reaction Conditions

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

Heat Inactivation

Incubation at 80℃ for 20 minutes.

Definition of Activity Unit

One unit of activity refers to the amount of enzyme required to completely digest 1 μg of λDNA in a 50 μl reaction system at 37℃ for 1 hour.

Quality Control Assays

Function

10 U of SgrAI can completely digest 1 μg of λDNA within 15 minutes at 37℃ .

Prolonged Incubation / Star Activity Assay

Under optimal reaction temperature, incubate 10 U SgrAI with 1 μg λDNA for 3 hours. No contamination from other nucleases or non-specific substrate degradation caused by star activity was detected. Longer incubation may result in star activity.

Ligation and Recuting

Under optimal reaction temperature, digest the substrate using 10 U BsrDI and recover the digested products. >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22℃ . Of these ligated fragments, >95% can be recut with BsrDI as determined by agarose gel electrophoresis.

Icon Descriptions

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

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

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

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

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

★ 3 hours incubation did not show star activity, but delayed enzyme digestion might show star activity.

Protocol

1. Protocol for DNA Digestion

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

ddH_2O	up to 50 μl
10× Cut Buffer B	5 μl
DNA[a]	1 μg
SgrAI (10 U/μl)	1 μl
Total	50 μl

a. DNA substrates should contain no phenol, chloroform, ethanol, EDTA, detergents, or high salt concentrations, otherwise enzyme activity will be affected;

② Mix gently and spin down.
③ Incubate at 37℃ for 15 minutes~1 hour.
④ Optional: Inactivate the enzyme by heating at 80℃ for 20 minutes, or by adsorption column or phenol/chloroform purification to terminate the reaction.

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

2. Notice

① Requires two or more sites for cleavage.
② Incubation of >10 units SgrAI/μg of DNA substrate is not recommended.
③ Based on the stability of the enzyme in the reaction, incubations longer than 1 hour will not result in improved digestion, unless additional enzyme is added.
④ Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%, overnight digestion is not recommended in particular.

Number of Recognition Sites in DNA

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

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	Impaired	Some blocked	Some blocked

Activity in Different Buffers*

	CutOne® Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart™ Buffer	Takara QuickCut™ Buffer
Activity	50%	＜ 12.5%	50%	＜ 12.5%

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

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