Tinzyme Co., Limited

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DraI

Product Number: DRA01

Shipping and Storage

Stored at -20ºC, valid for two years.

Components

Component	DRA01 200μl
DraI	200μl
10×Cut Buffer	0.8ml
Easy-Load 10×Cut Buffer	0.8ml

Description

DraI is a high-quality restriction endonuclease that has been genetically engineered and can quickly complete DNA cleavage using only one buffer within 5-15 minutes.Suitable for rapid enzymatic digestion of plasmid DNA, PCR products, or genomic DNA.

1. **Enzyme activity detection:** At the optimal reaction temperature, in a 20μl reaction system, 1μl DraI can completely digest 1μg of plasmid containing a single DraI cleavage site within 15 minutes.
2. **Long term enzyme digestion detection:** Incubate 1μl DraI with 1μg λDNA(HindIII digest) for 3 hours at the optimal reaction temperature, and no non-specific degradation of the substrate caused by other nucleases contamination or star activity was detected. Delayed enzyme digestion may result in star activity.
3. **Enzyme digestion ligation re digestion detection:** At the optimal reaction temperature, use 1μl DraI to digest the substrate, recover the enzyme digestion product, and use an appropriate amount of T4 DNA Ligase at 22 ℃ to reconnect the enzyme digestion product. After recovering the ligation product again, use the same endonuclease to cleave the ligation product again.
4. **Detection of non-specific endonuclease activity:** at the optimal reaction temperature, 1μl DraI and 1μg super spiral plasmid DNA were incubated together for 4h, and agarose gel electrophoresis was used to detect that the plasmid DNA was still in the super spiral state.
5. **Blue white spot detection:** The vector containing a single lacZα gene was digested with 1μl DraI, reconnected, and transformed into competent E. coli cells. The cells were then coated on LB medium plates containing corresponding antibiotics, IPTG, and X-gal. Products with correct connections will grow blue colonies, while products with incorrect connections (i.e. incomplete DNA end incisions) will grow white colonies. For our restriction enzyme, the proportion of white colonies should be less than 1%

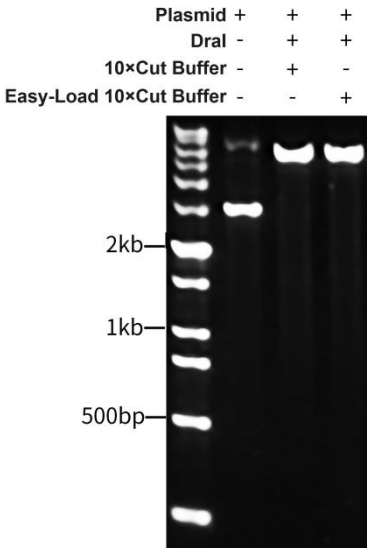

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Figure 1. Experimental results of DraI enzyme activity. In a 20μl reaction system, 1μg of pET-N-His-PreScission SUMO (5625bp) and 1μl of DraI were added or not added as shown in the figure. Enzyme cleavage reaction was performed using 1X Cut Buffer and 1X Easy Load Cut Buffer, respectively. The enzyme was incubated at 37°C for 15 minutes and inactivated at 65°C for 20 minutes. Then electrophoresis was performed and nucleic acid staining was performed using NA Red (EB upgraded product, 2000X), followed by fluorescence imaging analysis. The DNA marker used is DNA Ladder (0.2-12 kb, 12 bands). The actual detection effect may vary due to differences in experimental conditions, detection instruments, etc. The effect shown in the figure is for reference only.

Basic information

Recognition sequence	Isoschizomer	Enzyme digestion temperature	Deactivation conditions	Methylation interference?
5'-TTT^AAA-3' 3'-AAA^TTT-5'	AhaIII	37℃	65℃ 20min	Due to the influence of EcoKI methylation, the sequences completely overlap and are cut off

The activity (buffer compatibility) in different reaction buffers is as follows:

10×Cut Buffer	Easy-Load 10×Cut Buffer	Thermo FastDigest Buffer	NEB CutSmart® Buffer	Takara QuickCut™ Buffer
100%	100%	100%	100%	100%

Please refer to the table below for the methylation effects of DraI recognition sites:

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	No effect	No effect	Completely overlaps-blocked.	No effect

Features

1. Enzymatic cleavage can be completed within 5-15 minutes;
2. All endonucleases share a single enzyme digestion buffer, Cut Buffer, greatly simplifying the enzyme digestion reaction system and facilitating double or multiple enzyme digestion;
3. In response to the issue of differences in activity of different enzymes in Cut Buffer, the concentrations of different enzymes were adjusted to uniformly add 1μl of enzyme per 20μl of system for enzyme digestion reaction;
4. Many modifying enzymes, such as Alkaline Phosphatase, Antarctic Phosphatase, T4 DNA Ligase, T4 Polynucleotide Kinase, T4 PNK (3 'phosphatase minus), etc., are 100% compatible with Cut Buffer, making reaction systems such as "enzyme cut connect" and "enzyme cut modify connect" compatible and supporting single tube reactions;
5. Good enzyme activity redundancy makes it easy to cope with substrate excess or difficult template enzyme digestion.

Note

1. Endonucleases should be stored in an ice box or on an ice bath during use, and should be immediately stored at -20°C after use.
2. If it is found that the expected enzyme cleavage site cannot be cleaved, please confirm whether there is methylation interference.
3. Homolytic enzymes may have different sensitivities to different methylation modifications, and when encountering potential methylation interference issues, they can be attempted.
4. This product is only for scientific research by professionals and should not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residential areas.
5. For your safety and health, please wear lab coats and disposable gloves when operating.

Protocol

1. When performing single enzyme digestion, the following reaction system can be referred to and operated on an ice bath.

Reagent	Plasmid DNA	PCR Product	Genomic DNA
Ultrapure Water	(17-x)μl	(26-x)μl	(40-x)μl
10×Cut Buffer or Easy-Load 10×Cut Buffer	2μl	3μl	5μl

Substrate DNA	xμl(up to 1μg)	xμl(~0.2μg)	xμl(5μg)
DraI	1μl	1μl	5μl
Total volume	20μl	30μl	50μl
Incubate at 37ºC	15min	15-30min	30-60min

Note: The above reaction system is applicable for enzyme digestion of purified PCR products. The unpurified PCR product has a certain ionic strength and pH, and the amount of 10×Cut Buffer added can be appropriately reduced to 2μl. However, due to the simultaneous exonuclease activity of many DNA polymerases, it can affect the cleavage products. Therefore, the following steps require ligation, cloning, and other operations. It is recommended to purify the PCR products before cleavage.

1.1. After adding various liquids in sequence according to the table above, use a pipette to gently suck or tap the tube wall to mix (do not vortex), and then centrifuge instantly to settle the liquid to the bottom of the tube.

1.2. Incubate at 37ºC for 15 minutes (plasmid), 15-30 minutes (PCR product), or 30-60 minutes (genomic DNA). It is recommended to use a water bath as the preferred method for enzyme digestion reactions, as the reaction temperature is usually more constant.

1.3. Incubate at 65°C for 20 minutes to inactivate the enzyme and stop the reaction (optional).

2. When performing double or multiple enzyme digestion, the reaction system can be set according to the following principles based on the enzyme digestion reaction system settings in the reference form.

2.1. The dosage of each rapid endonuclease is 1μl, and the reaction system should be appropriately expanded as needed.

2.2. The total volume of all rapid endonucleases must not exceed 1/10 of the total reaction system.

2.3. If the optimal reaction temperatures for the several rapid endonucleases used are different, the enzyme with the lower optimal temperature should be used first for digestion, and then the enzyme with the higher optimal temperature should be added for digestion reaction at its optimal reaction temperature.

Related products

Product Number	Product Name
APA01	ApaLI
AS01	AscI
AV01	AvrII
BAM01	BamHI
BC01	BclI
BG01	BglII
BSA01	BsaI
BSN01	BspQI
BSN02	BstBI
BSN03	BstEII
CF01	Cfr9I
CL01	ClaI
DPN01	DpnI
DPN02	DpnII
DRA01	DraI
EA01	EagI
EA02	EarI
EC02	EcoRI
EC01	EcoRV
FSP01	FspI

HID01	HindIII
HIN01	HinfI
HPA01	HpaI
KAS01	KasI
KPN01	KpnI
ML01	MluI
RS02	MnlI
MSP01	MspI
NC01	NcoI
ND01	NdeI
NH01	NheI
NO01	NotI
NR01	NruI
NS01	NsiI
PA01	PacI
PM01	PmeI
PS01	PstI
PV01	PvuII
SA02	SacI
SA05	SacII
SA03	SalI
SA04	SapI
SB02	SbfI
SC02	ScaI
SF01	SfiI
SM01	SmaI
SP01	SpeI
SP02	SphI
SSP01	SspI
STE01	StuI
TAQ01	TaqI
XB01	XbaI
XC01	XcmI
XH01	XhoI
XM01	XmaI