LightNing® DpnI

REF: EG15585S





Isoschizomers*: MalI
Neoschizomers*: Bsp143I, BssMI, BstKTI, BstMBI, DpnII, Kzo9I, MboI, NdeII, Sau3AI

*Neoschizomers produce distinct ends, and isoschizomers/neoschizomers may exhibit different sensitivities to various methylation modifications.

Storage Condition

Store at -20℃ for 2 years.

Components

Components	Amount
LightNing® DpnI (20 U/μl)	50 μl (1000 U)
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

Description

LightNing® enzymes are a series of engineered restriction enzymes that are capable of fast DNA digestion. All LightNing® enzymes show superior activity in the universal CutOne® and CutOne® Color Buffer, and are able to digest DNA in 5~15 minutes. This enables any combination of restriction enzymes to work simultaneously in one reaction tube and eliminates the need for sequential digestions. LightNing® enzymes have passed multiple strict quality controls, and can be used to digest plasmid, genomic and viral DNA as well as PCR products.

CutOne® Color Buffer includes a density reagent along with red and yellow tracking dyes that allow for direct loading of the reaction mixtures on a gel. The red dye of the CutOne® Color Buffer migrates with 2.5 kb double-strand DNA fragments in a 1% agarose gel, and the yellow dye migrates with 10 bp double-strand DNA fragments in a 1% agarose gel.

Recommended Reaction Conditions

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

Heat Inactivation

Incubation at 65℃ for 5 minutes.

Quality Control

Functional Test

A 20 μl reaction in CutOne® Buffer containing 1 μg of pUC19 DNA and 1 μl of LightNing® DpnI 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 CutOne® Buffer containing 1 μg of pUC19 DNA and 1 μl of LightNing® DpnI 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.

Non-specific Endonuclease Activity

A 20 μl reaction in CutOne® Buffer containing 1 μg of supercoiled plasmid and 1 μl of LightNing® DpnI incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Icon Descriptions

- This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.
- 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.
- EB Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the EcoBI methylase.
- EPI Epimark-validated enzyme for epigenetic studies.
- The enzyme can be heat inactivated at by incubation 65℃ for 5 minutes.
- ★ 3 hours incubation do not show star activity, but longer incubation may result in star activity.

BestEnzymes Biotech Co., Ltd.
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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	Genomic DNA
ddH_2O	15 µl	30 µl
10× CutOne® Buffer or 10× CutOne® Color Buffer	2 µl	5 µl
DNA	2 µl (up to 1 µg)	10 µl (5 µg)
LightNing® DpnI	1 µl	5 µl
Total	20 µl	50 µl

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 5 minutes at 65℃ ;
⑤ If the CutOne® Color Buffer was used in the reaction, load an aliquot of the reaction mixture directly on a gel.

2. Double and Multiple Digestion of DNA

① Use 1 µl 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.

3. Scaling up Plasmid DNA Digestion Reaction

DNA	1 µg	2 µg	3 µg	4 µg	5 µg
LightNing® DpnI	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.

4. Digesting Plasmid Templates in PCR Products

Add 1 µl of LightNing® DpnI to 50 µl of PCR products, mix well, incubate at 37℃ for 60 minutes, and then heat-inactivate at 80℃ for 20 minutes. The resulting product can be used for downstream transformation experiments.

Number of Recognition Sites in DNA

λDNA	ΦX174	pBR322	pUC57	pUC18/19	SV40	M13mp18/19	Adeno2
116	0	22	15	15	8	7	87

Methylation Effects on Digestion

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

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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Add: No.17 Huaguoshan Avenue,Lianyungang City, China
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