LightNing® NcoI

REF: EG15550S





Isoschizomers*: Bsp19I

*Isoschizomers may have different methylation sensitivities.

Storage Condition

Store at -20℃ for 2 years.

Components

Components	Amount
LightNing® NcoI (20 U/μl)	30 μl (600 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 80℃ for 5 minutes.

Quality Control

Functional Test

A 20 μl reaction in CutOne® Buffer containing 1 μg of λDNA and 1 μl of LightNing® NcoI 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 λDNA and 1 μl of LightNing® NcoI 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.

Ligation and Recuting

After 10-fold over-digestion with LightNing® NcoI 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 LightNing® NcoI as determined by agarose gel electrophoresis.

Non-specific Endonuclease Activity

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

Blue/White Screening Assay

An appropriate vector containing *lacZα* gene is digested by 1 μl LightNing® NcoI. The digested product is ligated and transformed into *E.coli* competent cell. On Luria-Bertani culture plate with X-Gal, IPTG and appropriate antibiotic, the successfully ligated β-galactosidase gene can be expressed and gives rise to a blue colony, while an interrupted gene (i.e. degraded DNA end) gives rise to a white colony. LightNing® restriction enzymes must produce fewer than 1% white colonies.

Icon Descriptions

- This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.
- The enzyme's optimum reaction temperature is 37℃ .
- The enzyme can be heat inactivated at by incubation 80℃ for 5 minutes.
- 3 hours incubation do not show star activity, but longer incubation may result in star activity.

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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)
LightNing® NcoI	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 5 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. 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® NcoI	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
4	0	0	0	0	3	0	20

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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