

LightNing® HaeIII

REF: YS0171



Isoschizomers*: BsuRI, BshFI, BspANI, BsnI
*Isoschizomers may have different methylation sensitivities.

Storage Condition

-20°C

Components

Components	Amount
LightNing® HaeIII (10 U/μl)	300 μl (3000 U)
10× CutOne® Buffer	2×1 ml
10× CutOne® Color Buffer	2×1 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°C ;
Refer to "Protocol for Fast DNA Digestion" for reaction setup.

Heat Inactivation

Incubation at 80°C for 20 minutes.

Quality Control

Functional Test

A 20 μl reaction in CutOne® Buffer containing 1 μg of λDNA and 1 μl of LightNing® HaeIII incubated for 15 minutes at 37°C 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® HaeIII incubated for 3 hours at 37°C 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 Recutting

After 10-fold over-digestion with LightNing® HaeIII at 37 °C , >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22 °C . Of these ligated fragments, >95% can be recut with LightNing® HaeIII as determined by agarose gel electrophoresis.

Icon Descriptions

-  This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.
-  The enzyme's optimum reaction temperature is 37°C .
-  The enzyme can be heat inactivated at by incubation 80°C for 20 minutes.
-  3 hours incubation do not show star activity, but longer incubation may result in star activity.

All-in-One First-Strand Synthesis

MasterMix

REF: YS0147

Storage Condition

-20°C

Components

Component	Amount
All-in-One First-Strand Synthesis MasterMix	400 µl
Nuclease-Free Water	2×1 ml

Description

All-in-One First-Strand Synthesis MasterMix is a high-quality, efficient, and convenient one-step cDNA synthesis mix. It is designed to minimize contamination and contains all the necessary components for first-strand cDNA synthesis, including thermostable M-MLV GIII Reverse Transcriptase and its reaction buffer, RNase inhibitor, dNTPs, Oligo(dT)₂₀VN, and random primers—all the necessary components. Simply add RNA template and water to initiate the reaction. Using the reverse transcription pre-mix, cDNA of up to 12 kb in size can be obtained within 15 minutes.

Protocol

1. Prepare the following reaction mixture on ice:

Reagent	Amount
Template RNA ^a	50 ng~1 µg
All-in-One First-Strand Synthesis MasterMix	4 µl
Nuclease-Free Water	To 20 µl

a. It is recommended to use high-quality RNA extracted using a kit, which has been treated to remove genomic DNA contamination, as the template for the reaction.

- ② Mix gently and spin down.
- ③ Incubate at 55°C for 15 minutes.
[Note: If the template RNA does not contain a poly\(A\) tail, you may pre-incubate at 25°C for 10 minutes.](#)
- ④ Terminate the reaction by incubating at 85°C for 5 minutes.
- ⑤ Place the obtained cDNA product on ice for subsequent experiments.

Notice

The premix contains Oligo(dT)₂₀VN and random primers, suitable not only for eukaryotic mRNA containing poly(A) tail, but also for templates that do not contain poly(A) tail, such as prokaryotic RNA, eukaryotic rRNA, tRNA, etc. However, it is not suitable for small RNA like miRNA.

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 ₂ O	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® HaeIII	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°C 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°C ;
- 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® HaeIII	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
149	11	22	13	11	19	15	216

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.