LightNing® PspGI (EcoRII)

REF: EG15560S





Isoschizomers*: EcoRII, AjnI, Psp6I
Neoschizomers*: BciT130I, BseBI, Bst2UI, BstNI, MvaI
*Neoschizomers produce distinct ends, and isoschizomers/neoschizomers may exhibit different sensitivities to various methylation modifications.

Storage Condition

Store at -20℃ for 2 years.

Components

Component	Amount
LightNing® PspGI (10 U/μl)	200 μl (2000 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 75℃ ;
Refer to "Protocol for Fast DNA Digestion" for reaction setup.

Heat Inactivation

This enzyme can not be heat inactivated. Please purify the digested product by phenol/chloroform treatment or column-based purification kit.

Quality Control

Functional Test

A 20 μl reaction in CutOne® Buffer containing 1 μg of λDNA (Dcm-) and 1 μl of LightNing® PspGI incubated for 15 minutes at 75℃ 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 (Dcm-) and 1 μl of LightNing® PspGI incubated for 3 hours at 75℃ 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® PspGI at 75 ℃ , >95% of the DNA fragments can be ligated with T4 DNA Ligase at 22 ℃ . Of these ligated fragments, >95% can be recut with LightNing® PspGI as determined by agarose gel electrophoresis.

Non-specific Endonuclease Activity

A 20 μl reaction in CutOne® Buffer containing 1 μg of supercoiled plasmid and 1 μl of LightNing® PspGI incubated for 4 hours at 75℃ 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.
- The enzyme's optimum reaction temperature is 75℃ .
- Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the Dcm methylase.
- The enzyme can not be thermal inactivated.
- 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	42 µl	34 µl	30 µl
10× CutOne® Buffer or 10× CutOne® Color Buffer	5 µl	5 µl[a]	5 µl
DNA	2 µl (up to 1 µg)	10 µl (~0.2 µg)	10 µl (5 µg)
LightNing® PspGI	1 µl	1 µl	5 µl
Total	50 µl	50 µl	50 µl

a. For purified PCR products. If the PCR product is not purified and contains a certain ionic strength, the DNA polymerase may exhibit exonuclease activity, which can affect the enzyme digestion products. Therefore, for subsequent cloning or other operations, it is recommended to purify the PCR product before enzyme digestion..

② Mix gently and spin down;
③ Incubate at 75℃ 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℃ ;
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® PspGI	1 µl	2 µl	3 µl	4 µl	5 µl
10× CutOne® Buffer or 10× CutOne® Color Buffer	5 µl	5 µl	5 µl	5 µl	5 µl
Total	50 µl	50 µl	50 µl	50 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
71	2	6	5	5	17	7	136

Methylation Effects on Digestion

Dam	Dcm	CpG	EcoKI	EcoBI
No effect	Blocked	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%	50%

*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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