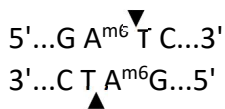


FlyCut™ DpnI

Cat #: C-BSM585

Size: 50 rxns

Storage: -20°C



Isoschizomers*: MspI

*Isoschizomers may have different methylation sensitivities.

Components

Components	Amount
FlyCut™ DpnI	50 µl
10× CutOne™ Buffer	1 ml
10× CutOne™ Color Buffer	1 ml

Description

FlyCut™ enzymes are a series of engineered restriction enzymes that are capable of fast DNA digestion. All FlyCut™ 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. FlyCut™ 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 pUC19 DNA and 1 µl of FlyCut™ DpnI 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 pUC19 DNA and 1 µl of FlyCut™ DpnI 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 FlyCut™ DpnI 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 FlyCut™ DpnI 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.
-  Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the CpG methylase.
-  Cleavage with this restriction enzyme may be blocked or impaired when the substrate DNA is methylated by the EcoBI methylase.
-  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.

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 ₂ O	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)
FlyCut™ DpnI	1 µl	5 µl
Total	20 µl	50 µl

- 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
FlyCut™ 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 µ	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
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 CutSmart® Buffer	Takara QuickCut™ Buffer
Activity	100%	100%	100%	100%

*The activity data come from the functional test described above.