

# AICON-BIO

## AICON-BIO™ DpnI

REF: EG15585S



Isoschizomers\*: Mall

\*Isoschizomers may have different methylation sensitivities.

## Storage Condition

-20°C

## Components

Components	Amount
AICON-BIO™ DpnI (20 U/μl)	50 μl (1000 U)
10× CutOne® Buffer	1 ml
10× CutOne® Color Buffer	1 ml

## Description

AICON-BIO™ enzymes are a series of engineered restriction enzymes that are capable of fast DNA digestion. All AICON-BIO™ 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. AICON-BIO™ 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 AICON-BIO™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 AICON-BIO™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.

### Non-specific Endonuclease Activity

A 20 μl reaction in CutOne® Buffer containing 1 μg of supercoiled plasmid and 1 μl of AICON-BIO™DpnI incubated for 4 hours at 37°C 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 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.

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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	Genomic DNA
ddH <sub>2</sub> O	15 µl	30 µl
10× CutOne <sup>®</sup> Buffer or 10× CutOne <sup>®</sup> Color Buffer	2 µl	5 µl
DNA	2 µl (up to 1 µg)	10 µl (5 µg)
AICON-BIO <sup>™</sup> Dpnl	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<sup>®</sup> 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
AICON-BIO <sup>™</sup> Dpnl	1 µl	2 µl	3 µl	4 µl	5 µl
10× CutOne <sup>®</sup> Buffer or 10× CutOne <sup>®</sup> 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
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 <sup>®</sup> Buffer	Thermo Scientific FastDigest Buffer	NEB rCutSmart <sup>™</sup> Buffer	Takara QuickCut <sup>™</sup> Buffer
Activity	100%	100%	100%	100%

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

## Activity of DNA Modifying Enzymes in CutOne<sup>®</sup> and CutOne<sup>®</sup> Color Buffers

EG15208S Alkaline Phosphatase (Fast)	100%
EG15205S T4 DNA Ligase (Fast)*	100%

\*ATP is required for T4 DNA Ligase activity.