

## Advantage of FlyCut® Restriction Enzymes

- Fast: 5 minutes DNA digestion.
- Simple: universal buffer for all restriction enzymes.
- High efficiency.
- No star activity.

## Protocol

### 1. Recommended Single Enzyme Digestion

Component	Volume	Final Concentration
DNA	Variable	≤1 µg
10×FlyCut® Buffer	2 µl	1×
FlyCut® Enzyme	0.5 µl	-
ddH <sub>2</sub> O	to 20 µl	-

Incubate at the suggested temperature for 5-15 minutes. To end digestion, add 10×DNA Loading Buffer to a final concentration of 1×, or heat to inactivate.

### 2. Recommended Double Enzyme Digestion (for enzymes digestion at the same temperature)

Component	Volume	Final Concentration
DNA	Variable	≤2 µg
10×FlyCut® Buffer	2 µl	1×
FlyCut® Enzyme I	0.5 µl	-
FlyCut® Enzyme II	0.5 µl	-
ddH <sub>2</sub> O	to 20 µl	-

Incubate at the suggested temperature for 5-15 minutes. To end digestion, add 10×DNA Loading Buffer to a final concentration of 1×, or heat to inactivate.

### 3. Recommended Double Enzyme Digestion (for enzymes digestion at different temperatures)

Component	Volume	Final Concentration
DNA	Variable	≤2 µg
10×FlyCut® Buffer	2 µl	1×
FlyCut® Enzyme I	0.5 µl	-
ddH <sub>2</sub> O	to 19.5 µl	-

Incubate at the suggested temperature for enzyme I for 5-15 minutes. Heat to inactivate enzyme I. Cool on ice. Add 0.5 µl of enzyme II, incubate at the suggested temperature for enzyme II for 5-15 minutes. Add 10×DNA Loading Buffer to a final concentration of 1×, or heat to inactivate.

## FlyCut® AvrII

JA101-01	50 units
JA101-02	100 units

### Storage

at -20°C for two years

### Concentration

5,000 units/ml

### Recognition Site

5'...CCTAGG...3'  
3'...GGATCC...5'

### Description

FlyCut® AvrII is expressed and purified from *E.coli* that carries the recombinant AvrII gene. The molecular weight is 42.3 kDa, with the recognition site at C<sup>^</sup>CTAGG. The reaction is optimized for 5-15 minutes at 37°C, and heat-inactivated at 80°C for 20 minutes. This enzyme is not sensitive to dam, dcm or mammalian CpG methylation.