

## FlyCut® PvuI

Please read the manual carefully before use.

Cat.No. JP201

Storage: at -20°C for two years

Concentration: 20,000 units/ml

### Description

*FlyCut*® PvuI is expressed and purified from *E.coli* that carries the recombinant PvuI gene. The molecular weight is 30.8 kDa, with the recognition site at CGAT<sup>^</sup>CG. The reaction is conducted for 5-15 minutes at 37°C, and heat-inactivated at 80°C for 20 minutes. This enzyme is not sensitive to dam, dcm, but sensitive to mammalian CpG methylation.

### Enzyme Properties

- Fast digestion in 5 minutes
- Universal buffer
- Star activity-free

### Application

Genomic DNA, plasmid DNA, PCR product

### Kit Contents

| Component                  | JP201-01  | JP201-02    |
|----------------------------|-----------|-------------|
| <i>FlyCut</i> ® PvuI       | 250 units | 2×250 units |
| 10× <i>FlyCut</i> ® Buffer | 250 µl    | 250 µl      |
| 10×DNA Loading Buffer      | 1 ml      | 1 ml        |

### Unit Definition

One unit is defined as the amount of enzyme required to digest 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 50 µl.

### Quality Control

**Ligation and re-cutting:** After 10-fold overdigestion with *FlyCut*® PvuI, more than 95% of the DNA fragments can be ligated with T4 DNA ligase at 25°C. Of these ligated fragments, more than 95% can be recut.

**16-Hour incubation:** A 50 µl reaction containing 1 µg of DNA and 10 units of enzyme incubated for 16 hours results in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

**Blue/White screening** (Terminal integrity): A DNA vector is digested at a unique site within lacZα gene with a 10-fold excess of enzyme, and then ligated, transformed and plated on X-gal/IPTG plate. Successful expression of the β-galactosidase indicates that lacZα gene remains integrity after cloning. A blue colony represents an intact gene, and a white colony represents an interrupted gene. To be Blue/White certified, enzymes must produce fewer than 3% white colonies.

**Exonuclease activity:** After incubation for 4 hours at 37°C, a 50 µl reaction containing 100 units of enzyme and 1 µg <sup>3</sup>H DNA releases less than 0.1% radioactive substance.

**Endonuclease activity:** After incubation for 4 hours at 37°C, a 50 µl reaction containing 15 units of enzyme with 1 µg pBR322 RFI DNA results in less than 10% conversion from RFI to RFII.

### Storage Buffer

20 mM Tris-HCl pH7.4, 250 mM NaCl, 0.1 mM EDTA, 1.5 mM DTT, 400 µg/ml BSA, 50% Glycerol

### 10×*FlyCut*® Buffer

500 mM Tris-Ac pH7.9, 1 M KAc, 120 mM MgAc<sub>2</sub>, 1 mg/ml BSA



#### Recommended single digestion reaction system

| Component                             | Volume   | Volume   |
|---------------------------------------|----------|----------|
| DNA                                   | ≤1 µg    | 1-2 µg   |
| 10× <i>FlyCut</i> <sup>®</sup> Buffer | 2 µl     | 5 µl     |
| <i>FlyCut</i> <sup>®</sup> PvuI       | 0.5 µl   | 1 µl     |
| Nuclease-free Water                   | Variable | Variable |
| Total volume                          | 20 µl    | 50 µl    |

Prior to use, please completely mix the *FlyCut*<sup>®</sup> Buffer.

Increase the volume of enzyme, in case of digestion of >2 µg DNA or incomplete digestion, but the total volume of enzyme should be less than 1/10 of the reaction system.

Different DNA will have different effects of enzyme digestion due to their different structures, so the reaction time can be adjusted according to the digestion effect.

#### Reaction conditions

Incubation for 5-15 minutes at 37 °C. Enzyme is inactivated by adding 10×DNA Loading Buffer to a final concentration at 1×, or by heating at 80°C for 20 minutes.

#### Recommended double digestion reaction system

| Component                             | Volume   |
|---------------------------------------|----------|
| DNA                                   | ≤2 µg    |
| 10× <i>FlyCut</i> <sup>®</sup> Buffer | 5 µl     |
| <i>FlyCut</i> <sup>®</sup> Enzyme I   | 1 µl     |
| <i>FlyCut</i> <sup>®</sup> Enzyme II  | 1 µl     |
| Nuclease-free Water                   | Variable |
| Total volume                          | 50 µl    |

Prior to use, please completely mix the *FlyCut*<sup>®</sup> Buffer.

Increase the volume of enzyme, in case of digestion of >2 µg DNA or incomplete digestion, but the total volume of enzyme should be less than 1/10 of the reaction system.

Different DNA will have different effects of enzyme digestion due to their different structures, so the reaction time can be adjusted according to the digestion effect.

#### Reaction conditions

Incubation for 5-15 minutes at the recommended reaction temperature. Enzyme is inactivated by adding 10×DNA Loading Buffer to a final concentration at 1×, or by heating.

If the two enzymes require different reaction temperatures, please refer to the “Notes for Double Digestion”.

#### Notes for Double Digestion

The *FlyCut*<sup>®</sup> Buffer ensures 100% activity for any two enzymes while minimizing star activity.

- Follow the recommended conditions for setting up the reaction system: The glycerol concentration in the reaction should be <5% to avoid star activity. For example, in a 50 µl reaction system, the total enzyme volume should not exceed 5 µl.
- Incubate at the recommended temperature and time, overnight incubation is not advised for double digestion.
- If the two enzymes require different reaction temperatures: Add the first enzyme and incubate at its recommended temperature (e.g., SmaI at 25°C), heat-inactivate the first enzyme before adding the second enzyme, then incubate at its optimal temperature.

**For research use only, not for clinical diagnosis**

Service telephone +86-10-57815020

Service email [custserv@transgenbiotech.com](mailto:custserv@transgenbiotech.com)

