

## Velocut PacI

Cat.No.:HYF280

### Product Description

Velocut enzymes are a series of engineered restriction enzymes that are capable of fast DNA digestion. All Velocut enzymes show superior activity in the universal Buffer and 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. Velocut enzymes have passed multiple strict quality controls, and can be used to digest plasmid, genomic and viral DNA as well as PCR products.

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 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.



### Components

| Components               | 25 rxns    |
|--------------------------|------------|
| Velocut PacI             | 25 $\mu$ L |
| 10 $\times$ Buffer       | 1 mL       |
| 10 $\times$ Color Buffer | 1 mL       |

### Storage

Store at -20°C.

### Heat Inactivation

Incubation at 80°C for 20 minutes.

## Notes

1. This enzyme will digest unit substrate in 5~15 minutes under recommended reaction conditions.
2. The enzyme's optimum reaction temperature is 37°C.
3. The enzyme can be heat inactivated at by incubation 80°C for 20 minutes.
4. 3 hours incubation do not show star activity, but longer incubation may result in star activity.

## Quality Control

### 1. Functional Test

A 20 µL reaction in Buffer containing 1 µg of pPacI DNA and 1 µL of Velocut PacI incubated for 15 minutes at 37°C results in complete digestion as determined by agarose gel electrophoresis.

### 2. Prolonged Incubation / Star Activity Assay

A 20 µL reaction in Buffer containing 1 µg of pPacI DNA and 1 µL of Velocut PacI 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.

### 3. Ligation and Recutting

After 10-fold over-digestion with Velocut PacI 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 Velocut PacI as determined by agarose gel electrophoresis.

### 4. Non-specific Endonuclease Activity

A 20 µL reaction in Buffer containing 1 µg of supercoiled plasmid and 1 µL of Velocut PacI incubated for 4 hours at 37°C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

## Instructions

### 1. Protocol for Fast DNA Digestion

- 1) Combine the following reaction components on ice in the order indicated:

| Components                            | Plasmid DNA       | PCR product     | Genomic DNA  |
|---------------------------------------|-------------------|-----------------|--------------|
| ddH <sub>2</sub> O                    | 15 µL             | 16 µL           | 30 µL        |
| 10× Buffer <i>or</i> 10× Color Buffer | 2 µL              | 3 µL*           | 5 µL         |
| DNA                                   | 2 µL (up to 1 µg) | 10 µL (~0.2 µg) | 10 µL (5 µg) |
| Velocut PacI                          | 1 µL              | 1 µL            | 5 µL         |
| <b>Total</b>                          | <b>20 µL</b>      | <b>30 µL</b>    | <b>50 µL</b> |

\*For purified PCR products. If the PCR products are not purified, amount of 10× Buffer should be reduced to 2 μL due to the remaining metal ions in the unpurified PCR products. We recommend to purify PCR products before digestion if it will be used for cloning, because the exonuclease activity of some DNA polymerases may alter the end of cleaved DNA.

- 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 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         |
|---------------------------------------|--------------|--------------|--------------|--------------|--------------|
| Velocut PaeI                          | 1 μL         | 2 μL         | 3 μL         | 4 μL         | 5 μL         |
| 10× Buffer <i>or</i> 10× Color Buffer | 2 μL         | 2 μL         | 3 μL         | 4 μL         | 5 μL         |
| <b>Total</b>                          | <b>20 μL</b> | <b>20 μL</b> | <b>30 μL</b> | <b>40 μL</b> | <b>50 μL</b> |

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 |
|------|-------|--------|-------|----------|------|------------|--------|
| 0    | 0     | 0      | 0     | 0        | 0    | 1          | 1      |

## Methylation Effects on Digestion

| Dam       | Dcm       | CpG       | EcoKI     | EcoBI     |
|-----------|-----------|-----------|-----------|-----------|
| No effect | No effect | No effect | No effect | No effect |

### Activity in Different Buffers\*

|          | Hyasen<br>Buffer | Thermo Scientific<br>FastDigest Buffer | NEB<br>rCutSmart™ Buffer | Takara<br>QuickCut™ Buffer |
|----------|------------------|----------------------------------------|--------------------------|----------------------------|
| Activity | 100%             | 100%                                   | 100%                     | 100%                       |

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