## Product Description

The Biori fast-cut series enzymes are fast restriction endonucleases produced using recombinant DNA technology, capable of completing site-specific DNA digestion within 5-15 min. They are suitable for rapid restriction digestion of a variety of DNA substrates, including plasmid DNA, PCR products, and genomic DNA. All fast-cut series enzymes retain 100% activity in the universal 10× Cut-Buffer, enabling simultaneous digestion with multiple restriction endonucleases in a single reaction. This eliminates the need for stepwise digestion and effectively streamlines the experimental workflow.

The recognition and cleavage sequences are listed below:

5'...C ↓ TTAAG...3'

3'...GAATT ↑ C...5'

## Components

| Components | BR1G153-01<br>100 T |
|---|---|
| AflⅡ | 100 μL |
| 10×Cut-Buffer | 1×1 mL |

## Storage

Store at -20±5°C.

## Application

Digestion of plasmid DNA, PCR products, and genomic DNA.

## Notes

1. For research use only. Not for use in clinical diagnosis.
2. Mix thoroughly before use. Avoid repeated freeze-thaw cycles.
3. The volume of restriction endonuclease added should not exceed one-tenth of the total reaction volume.
4. Digestion for longer than 3 h is not recommended, as prolonged incubation may lead to star activity.

## Protocol

1. Rapid DNA Digestion Protocol

1.1 Prepare the reaction mixture as follows (operate on ice):

| Components | Plasmid DNA | PCR Products | Genomic DNA |
|---|---|---|---|
| 10×Cut-Buffer | 2 μL | 3 μL* | 5 μL |
| AflⅡ | 1 μL | 1 μL | 5 μL |
| DNA Substrate | 1 μg | 0.2 μg | 5 μg |
| $ddH_2O$ | To 20 μL | To 30 μL | To 50 μL |

*The reaction may be scaled up or down proportionally as required. This reaction setup is intended for the digestion of purified PCR products. For unpurified PCR products, which retain salts and other reaction components, the amount of 10× Cut-Buffer may be reduced to 2 μL. However, as DNA polymerase exhibits exonuclease activity that may compromise the integrity of the digested ends, if the digested product is to be used for downstream applications such as cloning, the PCR product should be purified before digestion.

1.2 Gently flick the tube to mix, and briefly centrifuge to collect the contents at the bottom.

1.3 Incubate at 37°C for 15 min (plasmid DNA), 15-30 min (PCR products), or 30-60 min (genomic DNA).

2. Double or Multiple Digestions

2.1 The amount of each restriction enzyme should follow the table above. The reaction volume may be increased or decreased as needed; however, the volume of 10× Cut-Buffer should be adjusted accordingly to maintain a final concentration of 1×.

2.2 The combined volume of all restriction endonucleases must not exceed one-tenth of the total reaction volume.

2.3 If the restriction endonucleases chosen have different optimal reaction temperatures, the enzyme requiring the lower temperature should be used first for digestion, and then the enzyme with the higher optimal temperature should be added and the incubation continued at the higher temperature.

3. Methylation Sensitivity

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

4. Number of Recognition Sites in Selected DNA Molecules

| pUC18/19 | SV40 | M13mp18/19 | Adeno2 | λDNA | ΦX174 | pBR322 | pUC57 |
|---|---|---|---|---|---|---|---|
| 0 | 1 | 0 | 4 | 3 | 2 | 0 | 0 |

5. Enzyme Activity in Different Buffers

| Reaction Buffer | 10×Cut-Buffer | Thermo Scientific FastDigest Buffer | NEB rCutSmart™ Buffer | Takara QuickCut™ Buffer |
|---|---|---|---|---|
| Relative activity | 100% | 75% | 100% | 100% |

6. Inactivation Conditions

Incubate at 80°C for 20 min.