

AllBio EcoR V-Fast Cut



Cat. No. ABTGREE301
 Storage : at -20°C for two years
 Concentration : 20,000 units/ml

Recognition Site
 5'...GAT[▼]ATC...3'
 3'...CTAT[▲]AG...5'

Description

AllBio EcoR V-Fast Cut is expressed and purified from E.coli that carries the recombinant EcoR V gene. The molecular weight is 29.5 kDa, with the recognition site at GAT[▲]ATC. The reaction is conducted for 5-15 minutes at 37°C, and heat-inactivated at 65°C for 20 minutes. This enzyme is not sensitive to dam or dcm methylation, but sensitive to mammalian CpG methylation.

Enzyme Properties

Fast digestion in 5-15 minutes with high fidelity

Application

Genomic DNA, plasmid DNA, PCR product

Kit Contents

Component	ABTGREE301-01	ABTGREE301-02
AllBio EcoR V-Fast Cut	2,000 units	2×2,000 units
10×FastCut Buffer	500 ul	1 ml
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 AllBio EcoR V-Fast Cut, 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 ³H 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×FastCut Buffer

500 mM Tris-Ac pH7.9, 1 M KAc, 120 mM MgAc₂, 1 mg/ml BSA

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Reaction Components

Component	20 μ l Volume	50 μ l Volume
DNA	$\leq 1 \mu\text{g}$	1-2 μg
10 \times FastCut Buffer	2 μ l	5 μ l
AllBio EcoR V-Fast Cut	0.5 μ l	1 μ l
ddH ₂ O	to 20 μ l	to 50 μ l

For digestion of $>2 \mu\text{g}$ DNA, please adjust the amount of each component based on above table.

Reaction Condition

Incubation for 5-15 minutes at 37°C. Enzyme is inactivated by adding 10 \times DNA Loading Buffer to a final concentration at 1 \times , or by heating at 65°C for 20 minutes.

Notes

- Thaw the 10 \times FlyCut Buffer completely and mix well before use.
- Low ionic strength, high enzyme concentration, glycerol concentration $> 5\%$, or pH > 8.0 may result in star activity.