ZipCut™ Restriction Enzyme BamHI

Cat. # GMD032-1

	GMD032-1 (600Rxns)	GMD032-2 (3000Rxns)	Storage
ZipCut™ Restriction Enzyme BamHI	500 µl	500 µl x 5	-20℃
10× Reaction Buffer	1 ml×2	1 ml×2 x 5	-20℃
10× Reaction Color Buffer	1 ml×2	1 ml×2 x 5	-20℃

The ZipCut™ Restriction Enzyme BamHI is a rapid cutting endonucleases, a member of a series of restriction endonucleases that have undergone genetic engineering recombination and can accurately complete DNA cutting plasmid DNA, PCR products or genomic DNA, etc. in 5 to 15 minutes.

Procedure

1) Set up the DNA digestion reactions according to recommendations below.

	Plasmid DNA	PCR Product	Genomic DNA
10× Reaction Buffer OR 10× Reaction Color Buffer	2µl	3 µl	5µl
DNA	2 µl (up to 1 µg)	10 µl (~0.2 µg)	10 µl (5 µg)
ZipCut™ Restriction Enzyme BamHI	1µl	1µl	5µl
ddH2O	15µl	16µl	30µl
Total	20µl	30µl	50µl

Notes: A) For PCR products, we recommend digesting only purified PCR products. B) The Color Buffer enables direct loading of the digested DNA to gel without adding loading buffer. Both buffers can be used to set up restriction digestion reactions.

2) Mix by flicking the tube or pipetting the content up and down. Avoid vigorous vortexing.

3) Incubate at 37℃ for 15 min for plasmid DNA, 15~30 min for PCR product， or 30~60 min for genomic DNA.

4) Inactivate enzyme by incubating reactions at 80℃ for 20 min.

Notes on Double or multiple DNA digestion

1) Use no more than 1µl of each restriction enzyme.
2) The total volume of both or all restriction enzymes must be less then 1/10 of the total reaction volume.
3) If the enzymes in the same reaction require different reaction temperature, start the incubation at the lower temperature first followed by the higher temperature.

Quality Control testing

1) **Functional activity:** At the optimal reaction temperature, in a 20 µl reaction system, 1 µl of the endonuclease BamHI complete the reaction within 15 min.

2) **Star activity test:** At the optimal reaction temperature, 1 µl the endonuclease BamHI was incubated with 1 µg λDNA for 3 h, and no other nuclease contamination or star activity was detected.

3) **Digestion-ligation-re-digestion assay:** At 37°C, 1 µl of the endonuclease BamHI was used to digest the substrate. The digested product was purified. The purified DNA was ligated with a fast T4 DNA at 22°C. Results showed that the digested product could be re-ligated. After the ligation product was recovered again, the ligation product was subject to digestion again using the same endonuclease. This digestion was found to be successful too.

4) **Detection of non-specific endonuclease activity:** At 37°C, 1 µl the endonuclease BamHI was incubated with 1 µg supercoiled plasmid DNA for 4 h. The treated plasmid DNA was found to be still supercoiled when detected by electrophoresis.

5) **Blue and white selection assay**: A vector DNA containing a single lacZα gene was digested with 1 µl BamHI, re-ligated and transformed into E.coli. LB medium plates with antibiotic, IPTG and X-gal. Correctly ligated products are expected to result in blue colonies, while incorrectly ligated products (i.e. products with incomplete nicks) would result in white colonies. For the reaction series of restriction enzymes, the proportion of white colonies was found to be less than 1%.