

Restriction Endonuclease BamH I

From *Bacillus amyloliquefaciens* H

Cat. No. 10 220 612 001	1000 units (10 U/μl)
Cat. No. 10 567 604 001	2500 units (10 U/μl)
Cat. No. 10 656 275 001	10 000 units (10 U/μl)
Cat. No. 10 798 975 001	10 000 units, high concentration (40 U/μl)
Cat. No. 11 274 031 001	50 000 units, high concentration (40 U/μl)



Version 13

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Store at -15 to -25°C

Stability/Storage The undiluted enzyme solution is stable when stored at -15 to -25°C until the control date printed on the label. Do not store below -25°C to avoid freezing.

Sequence specificity BamH I recognizes the sequence G/GATCC and generates fragments with 5'-cohesive termini (1), which contain the same tetra-nucleotide sequence GATC as the cohesive termini of the fragments of Bcl I, Bgl II, Mbo I, Sau 3A or Xho II (2).

Compatible ends BamH I generates compatible ends to Bcl I, Bgl II, Nde II (Mbo I), Sau 3A and Xho II.

Enzyme with compatible ends	Recognition sequence	New sequence if BamH I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		BamH I - Enzyme	Enzyme - BamH I	
BamH I	G/GATCC	G/GATCC	G/GATCC	BamH I + Bst I
Bcl I	T/GATCA	G/GATCA	T/GATCC	Dpn I, Nde II, Sau 3A
Bgl II	A/GATCT	G/GATCT	A/GATCC	Dpn I, Nde II, Sau 3A, Xho II
Nde II	/G*AT°C	G/G*AT°C	/GATCC	Dpn I, Nde II, Sau 3A
Sau 3A	/G°AT*C	G/G°AT*C	/GATCC	Dpn I, Nde II, Sau 3A
Xho II	Pu/GATCPy	G/GATCPy	Pu/GATCC	BamH I, Dpn I, Nde II, Sau 3A, Xho II

Isoschizomers The BamH I is an isoschizomer of Bst I.

Methylation sensitivity BamH I is not inhibited by overlapping dam-methylation but is inhibited by the presence of 5- or 4-methylcytosine at the internal C residue as indicated (*).

Storage buffer 10 mM Tris-HCl, 300 mM KCl, 1 mM EDTA, 1 mM DTE, 0.01% (v/v) Polydocanol, 50% Glycerol (v/v), pH approx. 7.4 (4°C).

Supplied Incubation buffer (10x) 100 mM Tris-HCl, 1 M NaCl, 50 mM MgCl₂, 10 mM 2-Mercaptoethanol; pH 8.0 (at 37°C), (Δ SuRE/Cut Buffer B).

Activity in SuRE/Cut Buffer System Bold face printed buffer indicates the recommended buffer for optimal activity:

A	B	L	M	H
100%	100%	75-100%	100%	25-50%

Incubation temp. 37°C

Unit definition One unit is the enzyme activity that completely cleaves 1 μg λDNA in 1 h at 37°C in a total volume of 50 μl SuRE/Cut buffer B. 1 μg pBR322 DNA is digested completely by ca. 2 units of Bam H I on account of the larger number of cleavage sites per μg pBR322 DNA as compared to λDNA.

Typical experiment

Component	Final concentration
DNA	1 μg
10 × SuRE/Cut Buffer B	5.0 μl
Repurified water	Up to a total volume of 50 μl
Restriction enzyme	1 unit

Incubate at 37°C for 1 h.

Heat inactivation

Up to 10 Units BamH I / μg DNA can be heat-inactivated by 15 min incubation at 65°C, higher enzyme concentrations can no more be completely inactivated under these conditions.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
5	3	1	0	2	1	1	1

Activity in PCR buffer

Relative activity in PCR mix (Taq DNA Polymerase buffer) is 100%. The PCR mix contained λ target DNA, primers, 10 mM Tris-HCl (pH 8.3, 20°C), 50 mM KCl, 1.5 mM MgCl₂, 200 μM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

BamH I fragments obtained by complete digestion of 1 μg λDNA are ligated with 1 unit T4-DNA ligase in a volume of 10 μl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM MgCl₂, 5 mM Dithioerythritol, 1 mM ATP, pH 7.5 (20°C) resulting in >95% recovery of 1 μg λDNA fragments. Subsequent re-cutting with BamH I yields > 95% of the typical pattern of λDNA × BamH I fragments.

Troubleshooting

A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, ethanol, SDS, high levels of NaCl, metal ions (e.g., Hg²⁺, Mn²⁺) inhibit or alter recognition specificity of many restriction enzymes. Such compounds should be removed by ethanol precipitation followed by drying, before the DNA is added to the restriction digest reaction. Appropriate mixing of the enzyme is recommended.

Star activity

BamH I exhibits star activity under non-optimal conditions. The relaxed specificities of BamH I are GGATCN or G(R)ATCC. The relaxed specificity of BamH I can be induced by lowering the ionic conditions of the RE buffer, by increasing the glycerol conc. to > 5% or by using excess enzyme.

Quality control

Lot-specific certificates of analysis are available at www.lifescience.roche.com/certificates

Absence of unspecific endonuclease activity

1 μg λDNA is incubated for 16 h in 50 μl incubation buffer with excess of BamH I. The number of enzyme units which do not change the enzyme-specific pattern is stated in the certificate of analysis.

Absence of exonuclease activity

Approx. 5 μg [³H]-labeled calf thymus DNA are incubated with 3 μl BamH I for 4 h at 37°C in a total volume of 100 μl 50 mM Tris-HCl, 10 mM MgCl₂, 1 mM Dithioerythritol, pH approx. 7.5. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

- 1 Roberts, R. J. *et al.* (1977) *Nature* **265**, 82.
- 2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-248.
- 3 George, J.&Chirikjian, J.G.(1982) *Proc. Natl. Acad. Sci. USA* **79**, 2432.
- 4 Kolesnikov, V. A. *et al.* (1981) *FEBS Lett.* **132**, 101.
- 5 Rebase The Restriction Enzyme Database: <http://rebase.neb.com>
- 6 Benchmate: <http://www.roche-applied-science.com/benchmate>
- 7 Brooks, J.E. *et al.* (1989) "Cloning the *Bam*HI restriction modification system" *Nucl. Acids Res.* **17**, 979-997.
- 8 Brooks, J.E. *et al.* (1991) "Characterization of the cloned *Bam* HI restriction modification system: its nucleotide sequence, properties of the methylase, and expression in heterologous hosts" *Nucl. Acids Res.* **19**, 841-850.
- 9 Hwang, H.-Y. & Yim, J. (1994) "Sol I, a novel isoschizomer of *Bam*HI isolated from *Streptovorticillium olivovorticillatum*" *Nucl. Acids Res.* **22**, 2197.
- 10 Newman, M. *et al.* (1994) "Structure of restriction endonuclease *Bam*HI and its relationship to *Eco* RI" *Nature* **368**, 660-664.
- 11 Smith, L.A. & Chirikjian, J.G. (1979) "Purification and Characterization of Sequence-specific Endonuclease *Bam*HI" *J. Biol. Chem.* **254**, 1003-1006.
- 12 Ushay, H.M. *et al.* (1981) "Inhibition of the *Bam*HI Cleavage and Unwinding of pBR322 Deoxyribonucleic Acid by the Antitumor Drug cis-Dichlorodiammineplatinum(II)" *Biochem.* **20**, 3744-3748.

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Commonly used bacterial strains

Strain	Genotype
BL21	<i>E. coli</i> B <i>F</i> ⁻ <i>dcm</i> <i>ompT</i> <i>hsdS</i> (<i>r</i> _B - <i>m</i> _B -) <i>gal</i> (Studier, F.W. <i>et al</i> (1986) <i>J. Mol. Biol.</i> , 189 , 113.)
C600 ^e	<i>supE44</i> <i>hsdR2</i> <i>thi-1</i> <i>thr-1</i> <i>leuB6</i> <i>lacY1</i> <i>tonA21</i> ; (Hanahan, D. (1983) <i>J. Mol. Biol.</i> 166 , 557.)
DH5 α	<i>supE44</i> Δ (<i>lacU169</i> (ϕ 80d/ <i>lacZ</i> Δ M15)) <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i> ; (Hanahan, D. (1983) <i>J. Mol. Biol.</i> 166 , 557.)
HB101	<i>supE44</i> <i>hsdS20</i> <i>recA13</i> <i>ara-14</i> <i>proA2</i> <i>lacY1</i> <i>galK2</i> <i>rpsL20</i> <i>xyl-5</i> <i>mtl-1</i> ; (Hanahan, D., (1983) <i>J. Mol. Biol.</i> 166 , 557.)
JM108	<i>recA1</i> <i>supE44</i> <i>endA1</i> <i>hsdR17</i> <i>gyrA96</i> <i>relA1</i> <i>thi</i> Δ (<i>lac-proAB</i>); (Yanisch- Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
JM109	<i>recA1</i> <i>supE44</i> <i>endA1</i> <i>hsdR17</i> <i>gyrA96</i> <i>relA1</i> <i>thi</i> Δ (<i>lac-proAB</i>) <i>F</i> [<i>traD36proAB</i> ⁺ , <i>lacI</i> ^q <i>lacZ</i> Δ M15]; (Yanisch- Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
JM110	<i>rpsL</i> (<i>Str</i> ^r) <i>thr</i> <i>leu</i> <i>thi-1</i> <i>lacY</i> <i>galK</i> <i>gaT</i> <i>ara</i> <i>tonA</i> <i>tsx</i> <i>dam</i> <i>dcm</i> <i>supE44</i> Δ (<i>lac-proAB</i>) <i>F</i> [<i>traD36proAB</i> ⁺ , <i>lacI</i> ^q <i>lacZ</i> Δ M15]; (Yanisch- Perron, C. <i>et al.</i> , (1985) <i>Gene</i> 33 , 103.)
K802	<i>supE</i> <i>hsdR</i> <i>gal</i> <i>metB</i> ; (Raleigh, E. <i>et al.</i> , (1986) <i>Proc.Natl. Acad.Sci USA</i> , 83, 9070.; Wood, W.B. (1966) <i>J. Mol. Biol.</i> , 16 , 118.)
SURE ^r	<i>recB</i> <i>recJ</i> <i>sbc</i> <i>C201</i> <i>uvrC</i> <i>umuC</i> : <i>Tn5</i> (<i>kan</i> ^r) <i>lac</i> , Δ (<i>hsdRMS</i>) <i>endA1</i> <i>gyrA96</i> <i>thi</i> <i>relA1</i> <i>supE44</i> <i>F</i> [<i>proAB</i> ⁺ , <i>lacI</i> ^q <i>lacZ</i> Δ M15 <i>Tn10</i> (<i>tet</i> ^r); (Greener, A. (1990) <i>Stratagies</i> , 3 , 5.)
TG1	<i>supE</i> <i>hsd</i> Δ 5 <i>thi</i> Δ (<i>lac-proAB</i>) <i>F</i> [<i>traD36proAB</i> ⁺ , <i>lacI</i> ^q <i>lacZ</i> Δ M15]; (Gibson, T.J. (1984) <i>PhD Theses. Cambridge University, U.K.</i>)
XL1-Blue ^r	<i>supE44</i> <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA46</i> <i>thi</i> <i>relA1</i> <i>lac</i> <i>F</i> [<i>proAB</i> ⁺ , <i>lacI</i> ^q <i>lacZ</i> Δ M15 <i>Tn10</i> (<i>tet</i> ^r)]; (Bullock <i>et al.</i> , (1987) <i>BioTechniques</i> , 5, 376.)

Ordering Information

Product	Application	Packsize	Cat. No.
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at 15 - 25 °C.	Kit (40 DNA ligation)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt- ended DNA fragments.	100 U 500 units (1 U/ μ l)	10 481 220 001 10 716 359 001
rAPid Phosphatase	Dephosphorylation of 5'-phosphate residues from nucleic acids	1000 U 5000 U	04 898 133 001 04 898 141 001
rAPid Dephos and Ligation Kit	Dephosphorylation of nucleic acids.	40 reactions 160 reactions	04 898 117 001 04 898 125 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5'-phosphate residues from nucleic acids.	1000 U (20 U/ μ l)	11 097 075 001
Agarose MP	Multipurpose agarose for analytical and preparative electrophoresis of nucleic acids	100 g 500 g	11 388 983 001 11 388 991 001
Agarose LE	Separation of nucleic acids in the range 0.2 - 1.5 kbp	100 g 500 g	11 685 660 001 11 685 678 001
Agarose Gel DNA Extraction Kit	For the elution of DNA fragments from agarose gels.	1 Kit (max. 100 reactions)	11 696 505 001
High Pure PCR Product Purification Kit	Purification of PCR or enzymatic modification reaction (e.g. restriction digest)	50 purifications 250 purifications	11 732 668 001 11 732 676 001
SuRE/Cut Buffer Set for Restriction Enzymes	Incubation buffers A, B, L, M and H for restriction enzymes	1 ml each (10 \times conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5 \times 1 ml (10 \times conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5 \times 1 ml (10 \times conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5 \times 1 ml (10 \times conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5 \times 1 ml (10 \times conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5 \times 1 ml (10 \times conc. solution)	11 417 983 001
Water, PCR Grade	Specially purified, double-distilled, deionized, and autoclaved	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
BSA, special quality for molecular biology	Maintaining enzyme stability	20 mg (1 ml)	10 711 454 001

Changes to previous version

Editorial changes.

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