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)

Please see label for lot specific values.

Version October 2004

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 *Bam*H 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 *Bam*HI 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 *Bam*H I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		*Bam*H I - Enzyme	Enzyme – *Bam*H I	
***Bam*H I**	**G/GATCC**	**G/GATCC**	**G/GATCC**	***Bam*H 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	*Bam*H I, *Dpn* I, *Nde* II, *Sau* 3A, *Xho* II

Isoschizomers The *Bam*H I is an isoschizomer of *Bst* I.

Methylation sensitivity *Bam*H 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 $MgCl_2$, 10 mM 2-Mercaptoethanol; pH 8.0 (at 37°C), (≙ 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* HI 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**	2.5 µl
Repurified water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **37°C** for 1 h.

Heat inactivation Up to 10 Units *Bam*H 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 $MgCl_2$, 200 µM dNTPs, 2.5 U Taq DNA polymerase. The mix was subjected to 25 amplification cycles.

Troubleshooting A critical component is the DNA substrate.
Many compounds used in the isolation of DNA e.g. phenol, chloroform , EtOH, SDS, high levels of NaCl, metals (*e.g.*, Hg^{2+}, Mn^{2+}), inhibit or alter recognition specifity of many restriction enzymes.
Such compounds should be removed by EtOH precipitation followed by drying, before the DNA is added to the restriction digest reaction.
Appropriate mixing of the enzyme is recommended.

Check out the Restrictions Enzymes Frequently Asked Questions at http://www.roche-applied-science.com/support.

Star activity *Bam*H I exhibits star activity under non-optimal conditions. The relaxed specificities of *Bam*H I are GGATCN or G(R)ATCC. The relaxed specificity of *Bam*H 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

See data label for lot-specific values.

Absence of unspecific endonuclease activity 1 µg λDNA is incubated for 16 h in 50 µl incubation buffer with excess of *Bam*H I . The number of enzyme units which do not change the enzyme-specific pattern is stated under "Endo"

Absence of exonuclease activity Approx. 5 µg [^{3}H]- labeled calf thymus DNA are incubated with 3 µl *Bam*H I for 4 h at 37°C in a total volume of 100 µl 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM Dithioerythritol, pH approx. 7.5. The release of radioactivity is calculated as a percentage value of liberated to input radioactivity per unit of enzyme (stated under "Exo").

Ligation and recutting assay *Bam*H I fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 unit T4-DNA ligase (Cat. No. 10 481 220 001) in a volume of 10 µl by incubation for 16 h at 4°C in 66 mM Tris-HCl, 5 mM $MgCl_2$, 5 mM Dithioerythritol, 1 mM ATP, pH 7.5 (20°C).
The percentage of ligation and subsequent recutting with *Bam* HI yielding the typical pattern of λ · *Bam* HI fragments are determined and stated under "Lig" and "Rec".

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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 Streptoverticillium olivoverticillatum" *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.

Ordering Information

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Product	Application	Packsize	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky-end or blunt-end DNA fragments in just 5 min at 15-25°C.	Kit (40 DNA ligations)	11 635 379 001
T4 DNA Ligase	Ligation of sticky- and blunt ended DNA fragments.	100 U 500 units (1 U/µl)	10 481 220 001 10 716 359 001
Alkaline Phosphatase, shrimp	Dephosphorylation of 5'-phosphate residues from nucleic acids. Heat inactivation: 15 min at 65° C.	1000 U	11 758 250 001
Alkaline Phosphatase (AP), special quality for molecular biology	Dephosphorylation of 5´-phosphate residues from nucleic acids.	1000 U (20 U/µl)	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 LM-MP	Low melting point agarose allows enzymatic manipulations	50 g 100 g	11 441 345 001 11 441 353 001
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× conc. solutions)	11 082 035 001
SuRE/Cut Buffer A	Restriction enzyme incubation	5× 1 ml (10× conc. solution)	11 417 959 001
SuRE/Cut Buffer B	Restriction enzyme incubation	5× 1 ml (10× conc. solution)	11 417 967 001
SuRE/Cut Buffer H	Restriction enzyme incubation	5× 1 ml (10× conc. solution)	11 417 991 001
SuRE/Cut Buffer L	Restriction enzyme incubation	5× 1 ml (10× conc. solution)	11 417 975 001
SuRE/Cut Buffer M	Restriction enzyme incubation	5× 1 ml (10× 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

Printed Materials

You can view the following manuals on our website:

Laminated Buffer Chart
Lab FAQS "Find a Quick Solution"
Restriction Enzyme Ordering Guide
Molecular Weight Markers for Nucleic Acids
Poster "Rec. Sequences of Restriction Enzymes"

Commonly used bacterial strains

Strain	Genotype
BL21	*E. coli B F⁻ dcm ompT hsdS(r_B- m_B-) gal* (Studier, F.W. et al (1986) *J. Mol. Biol.*, **189,** 113.)
C600[e]	*supE44 hsdR2 thi-1 thr-1 leuB6 lacY1 tonA21*; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
DH5α	*supE44* Δ(*lac*U169 (ϕ80d*lac*ZΔM15) *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *rel*A1; (Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.)
HB101	*supE44 hsdS20 recA13 ara-14 proA2 lacY1 galK2 rpsL20 xyl-5 mtl-1*; (Hanahan, D., (1983) *J. Mol. Biol.* **166**, 557.)
JM108	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB); (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
JM109	*recA1 supE44 endA1 hsdR17 gyrA96 relA1 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lacI*^{q} *lacZ*ΔM15]; (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
JM110	*rpsL (Str*^{r}*) thr leu thi-l lacY galK galT ara tonA tsx dam dcm supE44 Δ(lac-proAB) F'[traD36proAB*$^+$ *, lacI*^{q} *lacZΔM15];* (Yanisch- Perron, C. et al., (1985) *Gene* **33**, 103.)
K802	*supE hsdR gal metB;* (Raleigh, E. et al., (1986) Proc.Natl. Acad.Sci USA, 83, 9070.; Wood, W.B. (1966) J. Mol. Biol., **16**, 118.)
SURE[r]	*recB recJ sbc C201 uvrC umuC::Tn5(kan*^{r}*) lac ,* Δ(hsdRMS) endA1 gyrA96 thi relA1 supE44 F'[proAB$^+$ lacI^q lacZΔM15 Tn10 (tet^r); (Greener, A. (1990) Stratagies, **3**, 5.)
TG1	*supE hsd Δ5 thi* Δ(*lac-pro*AB) F'[*traD36proAB*$^+$, *lacI*^{q} *lacZ*ΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue[r]	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lacI*^{q} *lacZ*ΔM15 Tn10 *(tet*^{r}*)*]; (Bullock et al., (1987) BioTechniques, 5, 376.)

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