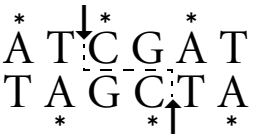
For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

Restriction Endonuclease Cla I

From Caryophanon latum L (1)

Cat. No. 10 404 217 001 500 units (10 U/µl)
Cat. No. 10 656 291 001 2500 units (10 U/µl)
Cat. No. 11 092 758 001 2500 units (40 U/µl)

Please see label for lot specific values.



Version Sept. 2004

Store at −15 to −25° C

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

Sequence specificity *Cla* I recognizes the sequence AT/CGAT and generates fragments with 5'CG-cohesive termini (1).

Compatible ends *Cla* I generates compatible ends to *Aci* I, *Acc* I, *Acy* I, *Hin* P1I, *Hpa* II, *Mae* II, *Msp* I, *Nar* I, *Psp* 1406 I, *Sfu I and Taq* I.

Enzyme with compatible ends	Recognition Sequence	Sequence if *Cla* I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		Cla I - Enzyme	Enzyme - Cla I	
Aci I	C/CGC	AT/CGC	CCGAT	–
Acc I	GT/(A,C)(T,G)AC	AT/CGAC	GT/CGAT	Taq I
Acy I	G(A,G)/CG(C,T)C	AT/CG(C,T)C	G(A,G)CGAT	–
***Cla* I**	**AT/CGAT**	**AT/CGAT**	**AT/CGAT**	***Cla* I + isoschizomers**
Hin P1I	G/CGC	ATCGC	GCGAT	–
Hpa II	C/CGG	ATCGG	CCGAT	–
Mae II	A/CGT	ATCGT	ACGAT	–
Msp I	C/CGG	ATCGG	CCGAT	–
Nar I	GG/CGCC	ATCGCC	GGCGAT	–
Psp 1406 I	AA/CGTT	ATCGTT	AACGAT	–
Sfu I	TT/CGAA	AT/CGAA	TT/CGAT	Taq I
Taq I	T/CGA	AT/CGA	T/CGAT	Taq I

Isoschizomers *Cla* I is an isoschizomer to *Ban* III, *Bsi* XI, *Bsp* 106 I, *Bsp* DI, *Bsu* 15

Methylation sensitivity *Cla* I is inhibited by overlapping dam-methylation (*). The single *Cla* I site located in the tetracycline resistance gene of pBR322 is not surrounded by dam-recognition sites and dam-inhibiting effects will not influence cloning experiments with pBR322. However, in other vectors inactivation by methylation of the *Cla* I sites may occur, unless the vectors are propagated in an E.coli dam$^-$ strain. *Cla* I is also inhibited by 5-methylcytosine as indicated (*).

Storage buffer 10 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, 0.02% polydocanol, 10 mM 2-Mercaptoethanol, 50% (v/v) Glycerol, pH approx. 7.5 (at 4° C).

Suppl. Incubation buffer (10x) SuRE/Cut Buffer **H**: 0.5 M Tris-HCl, 1 M NaCl, 100 mM $MgCl_2$, 10 mM DTE, pH 7.5 (at 37° C)

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%	**100%**

Incubation temperature **37° C**

Unit definition One Unit is the enzyme activity that completely cleaves 1 µg λ DNA in 1 h at **37° C** in the SuRE/Cut Buffer **H** in a total volume of 25 µl.

Typical experiment

Component	Final concentration
DNA	1 µg
10 × SuRE/Cut buffer **H**	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 *Cla* I is not heat-inactivated by 15 min incubation at 65° C.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
15	2	0	0	2	1	1	0

PFGE tested *Cla* I has been tested in Pulsed-Field Gel Electrophoresis (test system bacterial chromosomes). For cleavage of genomic DNA (E. coli C 600) embedded in agarose for PFGE analysis 10 units of enzyme/µg DNA and 4 h incubation time are recommended.

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.

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Quality Control See label for lot specific values

Absence of unspecific endonucleases 1µg λ DNA is incubated for 16 h in 50 µl SuRE/Cut Buffer H with excess of *Cla* I. The number of enzyme units which do not change the enzyme-specific pattern is stated under "Endo".

Absence of exonuclease Approx. 5 µg [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Cla* 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 enzmye (stated under "Exo").

Typical ligation and recutting assay *Cla* I fragments obtained by complete digestion of 1µg λ DNA are ligated with 1 U T4 DNA Ligase (Cat. No. 481 220) 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 (at 20° C). The percentage of ligation and subsequent recutting with *Cla* I which yields the typical pattern of λ × *Cla* I fragments is determined and stated under "Lig" and "Rec".

References

1 Mayer, H. et al. (1981) *Nucleic Acids Res.* **9**, 4833.
2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1-250.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://www.roche-applied-science.com/benchmate
5 Zieger, M. et al. (1987) "Two restriction endonucleases from Bacillus sphaericus: Bsp XI and Bsp XII" Nucl. Acids Res. **15,** 3919;

Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research.

For a complete overview of related products and manuals, please visit and bookmark our Special Interest Sites: http://www.roche-applied-science.com

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*lacZ*ΔM15) *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* Δ*5 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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