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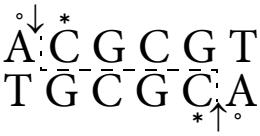
Not for use in diagnostic procedures.

Restriction Endonuclease Mlu I

From *Micrococcus luteus*



Cat. No. 10 909 700 001 500 units (10 U/µl)
Cat. No. 10 909 718 001 2500 units (10 U/µl)
Cat. No. 11 207 601 001 2500 units, high concentration (40 U/µl)

Version 18
Content version: February 2012
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 *Mlu* I recognizes the sequence A/CGCGT and generates fragments with 5´-cohesive termini (1).

Compatible ends *Mlu* I generates compatible ends to *Bss* HII.

Enzyme with compatible ends	Recognition sequence	New sequence if *Mlu* I is ligated to enzyme with compatible ends		Enzyme that can cut this new sequence
		Mlu I - Enzyme	Enzyme – *Mlu* I	
Bss HII	G/CGCGC	A/CGCGC	G/CGCG	*Acc* II, *Bst* UI, *Fnu* DII, *Mvn* I, *Tha* I
***Mlu* I**	**A/CGCGT**	**A/CGCGT**	**A/CGCGT**	***Afl* III, *Mlu* I, *Mvn* I**

Isoschizomers The enzyme is not known to have isoschizomers.

Methylation sensitivity *Mlu* I is inhibited at A*CGCGT by the presence of 5´-methylcytosine. *Mlu* I is not influenced by the presence of N^6-methyladenine (°).

Storage buffer 20 mM Tris-HCl, 250 mM KCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.2% Triton X-100, 50% Glycerol (v/v), pH approx. 8.0 (at 4° C).

Suppl. Incubation buffer 10x 500 mM Tris-HCl, 1 M NaCl, 100 mM $MgCl_2$, 10 mM Dithioerythrythol, pH 7.5 (at 37° C), (≙ SuRE/Cut Buffer **H**).

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

A	B	L	M	**H**
10-25%	25-50%	0-10%	10-25%	**100%**

Incubation temperature **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 25 µl SuRE/Cut buffer **H.**

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 There is no information about *Mlu* I and heat inactivation available.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
7	5	0	2	0	0	0	0

λDNA has 7 cleavages sites for *Mlu* I at following positions: 458, 5548, 15372, 17791, 19996, 20952 and 22220.

PFGE tested *Mlu* 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 < 5%. 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.

Ligation and recutting assay *Mlu* I fragments obtained by complete digestion of 1 µg λDNA 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 Dithiothreitol, 1 mM ATP, pH 7.5 (at 20° C) resulting in >95 % recovery of 1 µg λDNA fragments. Subsequent re-cutting with *Mlu* I yields > 95% of the typical pattern of λDNA × *Mlu* I fragments.

Troubleshooting A critical component is the DNA substrate. Many compounds used in the isolation of DNA such as phenol, chloroform, EtOH, SDS, high levels of NaCl, metals (*e.g.* Hg^{2+}, Mn^{2+}) inhibit or alter recognition specificity 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.

Quality control

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

Absence of unspecific endonuclease activities 1 µg λDNA is incubated for 16 h in 50 µl SuRE/Cut buffer H with excess of *Mlu* 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 [^{3}H] labeled calf thymus DNA are incubated with 3 µl *Mlu* 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. Under these conditions, no release of radioactivity is detectable, as stated in the certificate of analysis.

References

1 Sugisaki, H. & Kanazawa, S . (1991) *Gene* **16**, 73–78.
2 Kessler, C. & Manta, V. (1990) *Gene* **92**, 1–248.
3 Rebase The Restriction Enzyme Database: http://rebase.neb.com
4 Benchmate: http://roche-applied-science.com/benchmate

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 home page, www.roche-applied-science.com, and our Special Interest Sites, including "Mapping & Cloning": http://www.restriction-enzymes.com.

The convenient RE Finder Program located on our Bench Mate website, http://www.roche-applied-science.com/benchmate helps you identify the enzymes that will cut your DNA sequence, and displays the names and recognition sequences of enzymes and isoschizomers as well as links to detailed information (*e.g.* instructions for use) of the selected restriction enzyme.

Product	Application	Packsize	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website or catalogue	
Rapid DNA Ligation Kit	Ligation of sticky- or blunt-ended DNA fragments in just 5 min at +15 to +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
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/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 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× 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:

Lab FAQS "Find a Quick Solution"
Restriction Enzyme Ordering Guide
Molecular Weight Markers for Nucleic Acids

Changes to previous version Update of quality control.

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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*$^+$, *lac*I^q *lac*ZΔM15]; (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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