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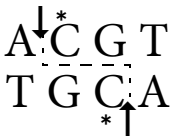
Restriction Endonuclease *Mae* II

From *Methanococcus aeolicus* PL-15/H

Cat. No. 10 862 495 001 50 units (1 - 5 U/µl)



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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 expiration date printed on the label. Do not store below −25 °C to avoid freezing.
Note: Product is shipped on dry ice.

Sequence specificity *Mae* II recognizes the sequence A/CGT and generates fragments with 5´-cohesive termini (1).

Compatible ends The enzyme generates compatible ends to *Acy* I, *Asu* II, *Cla* I, *Hind* I, *Hpa* II, *Msp* I, *Nar* I, *Sfu* I, and *Taq* I.

Isoschizomers *Mae* I is not known to have isoschizomers.

Methylation sensitivity *Mae* II is inhibited by the presence of 5-methylcytosine as indicated (*).

Storage buffer 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 200 µg/ml bovine serum albumin, 0.01% Polydocanol, 0.01 % Gelatine, 50% Glycerol (v/v), pH approx. 7.8 (at 4 °C).

Incubation buffer (2x, included) 100 mM Tris-HCl, 440 mM NaCl, 12 mM $MgCl_2$, 14 mM 2-Mercaptoethanol, pH 8.8 (at 50 °C)
We recommend to use the special incubation buffer supplied with the enzyme.

Activity in SuRE/Cut Buffer System Please use the supplied 2× special incubation buffer for *Mae* II, since the following activities were observed with the SuRE/Cut buffer system:

A	B	L	M	H
0-10	25-50	0-10	25-50	75-100

Special Incubation temp. **50 °C**

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 h **at 50 °C** in a total volume of 25 µl incubation buffer.

Typical experiment

Component	Final concentration
DNA	1 µg
2 × *Mae* II Buffer	12.5 µl
Sterile redist. water	Up to a total volume of 25 µl
Restriction enzyme	1 unit

Incubate at **50 °C** for 1 h.

Heat Inactivation There is no information about heat inactivation available.

Number of cleavage sites on different DNAs (2):

λ	Ad2	SV40	Φ X174	M13mp7	pBR322	pBR328	pUC18
143	83	0	19	22	10	12	5

Activity in PCR buffer Relative activity in PCR mix (Taq DNA Polymerase buffer) is **0%**. The PCR mix contained λtarget 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 *Mae* II fragments obtained by complete digestion of 1 µg λDNA are ligated with 1 U 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 dithiothreitol, 1 mM ATP, pH 7.5 (at 20 °C) resulting in >95 % recovery of 1 µg λDNA fragments.
Subsequent re-cutting with *Mae* II yields > 95% of the typical pattern of λDNA × *Mae* II 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.*, Hg^2, Mn^{2+}) 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.

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

Absence of unspecific endonuclease activities 1 µg λ DNA is incubated for 16 h in 50 µl incubation buffer with excess of *Mae* II. 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 *Mae* II 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 Schmid, K., *et al.* (1984) *Nucleic Acids Res.* **12**, 2619.
2 Kessler, C. & Manta, V. (1990) *Gene* **92,** 1–250.
3 Molloy, P.L. & Watt, F. (1988) *Nucl. Acids Res.* **16,** 2335.
4 Rebase The Restriction Enzyme Database: http://rebase.neb.com

Ordering Information

Product	Application	Packsize	Cat. No.
Restriction Enzymes	DNA restriction digestion	Please refer to website	
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
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

Changes to previous version

Editorial changes

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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*$^+$, *lac*I^q *lac*ZΔM15]; (Gibson, T.J. (1984) PhD Theses. Cambridge University, U.K.)
XL1-Blue^r	*supE44 hsdR17 recA1 endA1 gyrA46 thi relA1 lac* F'[*proAB*$^+$, *lac*I^q *lac*ZΔM15 Tn10 *(tet^r)*]; (Bullock *et al.,* (1987) BioTechniques, 5, 376.)

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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany