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Restriction Endonuclease *Mae* IL

From *Methanococcus aeolicus* PL-15/H

Cat. No. 10 822 221 001 250 units (1-5 U/µl)

Version 20
Content version: July 2017

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.
Note: The product is shipped on dry ice.

Sequence specificity *Mae* I recognizes the sequence C/TAG and generates fragments with 5'-cohesive termini (1).

Compatible ends The enzyme generates compatible ends to *Asn* I, *Nde* I, *Tru9* I (*Mse* I).

Isoschizomers *Mae* I is an isoschizomer to *Bfa* I.

Methylation sensitivity No information available.

Storage buffer 20 mM Tris-HCl, 100 mM NaCl, 0.1 mM EDTA, 10 mM 2-Mercaptoethanol, 0.01% Gelatine, 0.01% Polydocanol, 50% Glycerol (v/v), pH approx. 8.0 (at 4 °C).

Incubation buffer (2x, included) 40 mM Tris-HCl, 500 mM NaCl, 12 mM $MgCl_2$, 14 mM 2-Mercaptoethanol, pH 8.0 (at 45 °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* I, since the following activities were observed with the SuRE/Cut buffer system:

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

Incubation temperature **45 °C**

Unit definition One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 h at **45 °C** in the incubation buffer in a total volume of 25 µl.

Typical experiment

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

Incubate at **45 °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
14	54	12	3	4	5	4	4

Activity in PCR buffer Relative activity in PCR mix (Taq DNA Polymerasebuffer) is 0%. The PCR mix contained lambda 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 amplificationcycles.

Ligation and recutting assay *Mae* I fragments obtained by complete digestion of 1 µg pBR322 DNA 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 pBR322 DNA fragments. Subsequent re-cutting with *Mae* I yields > 95% of the typical pattern of pBR322 DNA × *Mae* 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.*, 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 λDNA is incubated for 16 h in 50 µl incubation buffer with excess of *Mae* 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 *Mae* 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 Schmid, K. *et al.* (1984) *Nucleic Acids Res.* **12**, 2619
2 Kessler, C. & Manta, V. (1990) *Gene* **92,** 1-248.
3 Sowers, K.R. (1995) in Archaea: Methanogens: A laboratory manual, pp 505-506.
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*$^+$, *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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