MmeI

Mme I

Restriction Endonuclease

Recognition Sequence:

5`-T C C R A C (N)$_{20}$-3`
3`-A G G Y T G (N)$_{18}$-5`

Cat. No.	Size
E2288-01	100 units
E2288-02	500 units

Reaction Temperature: 37˚C

Inactivation Temperature (20 min): 80˚C

Prototype: MmeI

Source: *Methylophilus methylotrophus*

Package Contents:
- → MmeI
- → 10x Reaction Buffer MmeI

Storage Conditions: Store at -20˚C.
Store reaction buffer in aliquots at -70˚C.

Double Digestion – Buffer Compatibility:

Buffer	% Relative Activity
Low	NR***
Medium	NR***
High	NR***
Acet	NR***

*** NR - buffer is not recommended, use 1 x buffer MmeI.

Recommended Buffer: MmeI

DNA Methylation:
No inhibition: dam, dcm, EcoKI
Inhibition (Blocked): CpG

Standard Reaction Protocol:

Mix the following reaction components:
- 1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
- 3 µl 10x Buffer MmeI
- 1-2 U MmeI (use 1 U / µg DNA, < 10 % React. Volume!)
  Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
- @ 30 µl H_2O, DNA and DNase free

Incubate for 1 h at 37˚C

Stop reaction by alternatively
- (a) Addition of 1.2 µl EDTA pH 8.0 [0.5 M], final 20 mM or
- (b) Heat Inactivation
  20 min at 80˚C or
- (c) Spin Column DNA Purification
  (e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) or
- (d) Gel Electrophoresis and Single Band Excision
  (e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) or
- (e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Unit Definition:

One unit is the amount of enzyme required to digest 1 µg of pUC19 DNA to obtain stable digestion pattern in 1 hr in a total reaction volume of 30 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x MmeI Buffer: 6 mM Tris-HCl (pH 7.5 at 25˚C), 6 mM $MgCl_2$, 2 mM dithiothreitol,+ enhancers.
Avoid multiple cycles of freezing/thawing of the stock reaction buffer /no more than 3 times/. Thawing should be performed at temperatures not exceeding 10°C. Recommended procedure is to divide the provided reaction buffer into smaller portions and preserve them at -70°C for long-term. Temperature of -20°C should be used only for short-term storage.
Note 1: Excess MmeI blocks cleavage.
Note 2: Blocked by overlapping CpG methylation.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 25˚C), 200 mM KCl, 1 mM EDTA, 5 mM beta-mercaptoethanol, 50 % [v/v] glycerol.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities.