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## roboklon

# Mmel

### 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 Mmel

- 1-2 U MmeI (use 1 U /  $\mu g$  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 H₂O, 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  $\mu g$  of pUC19 DNA to obtain stable digestion pattern in 1 hr in a total reaction volume of 30  $\mu l.$  Enzyme activity was determined in the recommended reaction buffer.

#### **Reaction Buffer:**

1~x~Mmel~Buffer: 6 mM Tris-HCl (pH 7.5 at 25°C), 6 mM MgCl\_z, 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 Mmel 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.

Mme I Restriction Endonuclease

Recognition Sequence:

#### 5`-T C C R A C (N)<sub>20</sub>-3` 3`-A G G Y T G (N)<sub>18</sub>-5`

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

#### Reaction Temperature: 37°C

## Inactivation Temperature (20 min): 80° C

## Prototype: Mmel

Source: Methylophilus methylotrophus

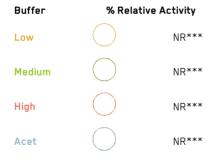
#### Package Contents:

→ Mmel

→ 10x Reaction Buffer Mmel

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

## Double Digestion - Buffer Compatibility:



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

### Recommended Buffer: Mmel

#### **DNA Methylation:**

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