

CERTIFICATE OF ANALYSIS

# MreI (Sse232I)

#ER2021      300 u

Lot:                      Expiry Date:

5'...**C G↓C C G G C G**...3'

3'...**G C G G C C↑G C**...5'

Concentration:      10 u/μl  
Source:                *E.coli* that carries the cloned *mreI*R  
gene from *Micromonospora*  
*rhodorangea* RFL1

Supplied with:      1 ml of 10X Buffer G  
1 ml of 10X Buffer Tango™

Store at -20°C



In total 3 vials.

BSA included: Lot# BSA62-313P

## RECOMMENDATIONS

**1X Buffer G** (for 100% MreI digestion)

10 mM Tris-HCl (pH 7.5), 10 mM MgCl<sub>2</sub>, 50 mM NaCl,  
0.1 mg/ml BSA.

**Incubation temperature**

37°C.

**Unit Definition**

One unit is defined as the amount of MreI required to digest 1 μg of control DNA in 1 hour at 37°C in 50 μl of recommended reaction buffer. The control DNA is linearized pJET1 DNA with inserted MreI recognition site.

**Dilution**

Dilute with Dilution Buffer (#B19): 10 mM Tris-HCl (pH 7.4 at 25°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 0.2 mg/ml BSA and 50% glycerol.

**Double Digests**

Tango™ Buffer is provided to simplify buffer selection for double digests. 98% of Fermentas restriction enzymes are active in a 1X or 2X concentration of Tango™ Buffer. Please refer to the Fermentas Catalog or go to [www.fermentas.com/doubledigest](http://www.fermentas.com/doubledigest) to choose the best buffer for your experiments.

1X Tango™ Buffer:

33 mM Tris-acetate (pH 7.9 at 37°C), 10 mM magnesium acetate, 66 mM potassium acetate, 0.1 mg/ml BSA.

## Storage Buffer

Mrel is supplied in: 10 mM potassium phosphate (pH 7.0 at 25°C), 200 mM KCl, 0.1 mM EDTA, 0.01% Triton X-100, 0.2 mg/ml BSA and 50% glycerol.

## Recommended Protocol for Digestion

- Add:

nuclease-free water	16 µl
10X Buffer G	2 µl
DNA (0.5-1 µg/µl)	1 µl
Mrel	0.5-2 µl
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

The digestion reaction may be scaled either up or down.

## Recommended Protocol for Digestion of PCR Products Directly after Amplification

- Add:

PCR reaction mixture	10 µl	(~0.1-0.5 µg of DNA)
nuclease-free water	18 µl	
10X Buffer G	2 µl	
Mrel	1-2 µl	
- Mix gently and spin down for a few seconds.
- Incubate at 37°C for 1-16 hours.

## Thermal Inactivation

Mrel is inactivated by incubation at 80°C for 20 min.

## ENZYME PROPERTIES

### Enzyme Activity in Fermentas REase Buffers, %

B	G	O	R	Tango™	2X Tango™
20-50	100	0-20	0-20	50-100	0-20

### Methylation Effect on Digestion

Dam: never overlaps – no effect.  
Dcm: never overlaps – no effect.  
CpG: completely overlaps – blocked.  
EcoKI: never overlaps – no effect.  
EcoBI: never overlaps – no effect.

### Stability during Prolonged Incubation

A minimum of 0.5 units of the enzyme is required for complete digestion of pJET1 DNA with inserted Mrel recognition sequence in 16 hours at 37°C.

### Compatible Ends

BshTI, Cfr9I, SgrAI, BseNI, Cfr10I, Kpn2I, BsaWI.

### Number of Recognition Sites in DNA

λ	ΦX174	pBR322	pUC57	pUC18/19	pTZ19R/U	M13mp18/19	Ad2
0	0	0	0	0	0	0	2

For **QUALITY CONTROL ASSAY DATA** see back page

## QUALITY CONTROL ASSAY DATA

### Overdigestion Assay

No detectable change in the specific fragmentation pattern is observed after a 160-fold overdigestion with Mrel (10 u/μg DNA x 16 hours).

### Ligation/Recutting Assay

After a 50-fold overdigestion (3 u/μg DNA x 17 hours) with Mrel, more than 95% of the digested DNA fragments can be ligated at a 5'-termini concentration of 0.1 μM. More than 95% of these sites can be recut.

### Labeled Oligonucleotide (LO) Assay

No detectable degradation of single-stranded or double-stranded labeled oligonucleotides occurred during incubation with 10 units of Mrel for 4 hours.

### Blue/White Cloning Assay

A mixture of pUC57/HindIII, pUC57/Eco32I and pUC57/PstI digests was incubated with 10 units of Mrel for 16 hours. After religation and transformation, the background level of white colonies was 0.2%.

Quality authorized by:

 Jurgita Zilinskiene

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*Certain countries are out of the scope of patent coverage.*

### PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to [www.fermentas.com](http://www.fermentas.com) for Material Safety Data Sheet of the product.

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