

# TspMI



1-800-632-7799  
info@neb.com  
www.neb.com



R0709S 003121113051

## R0709S



200 units Lot: 0031211 Exp: 5/13

5,000 U/ml Store at -20°C

### Recognition Site:

5'... C<sup>▼</sup>CCGGG ... 3'  
3'... GGGCC<sup>▲</sup>C ... 5'

Source: *Thermus* species (P. Sharma)

Supplied in: 300 mM NaCl, 20 mM Tris-HCl  
(pH 8.0 @ 25°C), 1 mM EDTA, 1 mM DTT, 0.1%  
Triton X-100, 200 µg/ml BSA and 50% glycerol.

### Reagents Supplied with Enzyme:

10X NEBuffer 4.

### Reaction Conditions:

1X NEBuffer 4  
Incubate at 75°C.

### 1X NEBuffer 4:

20 mM Tris-acetate  
10 mM magnesium acetate  
50 mM potassium acetate  
1 mM dithiothreitol  
pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to digest 1 µg of pUCAdeno plasmid DNA in 1 hour at 75°C in a total reaction volume of 50 µl.

### Diluent Compatibility:

Diluent Buffer B  
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA,  
1 mM dithiothreitol, 500 µg/ml BSA and 50%  
glycerol (pH 7.4 @ 25°C).

### Quality Control Assays

**Ligation:** After 15-fold overdigestion with TspMI,  
> 95% of the DNA fragments can be ligated with  
T4 DNA Ligase (at a 5' termini concentration

of 1–2 µM) at 16°C. Of these ligated fragments,  
> 95% can be recut.

**16-Hour Incubation:** A 50 µl reaction containing  
1 µg of DNA and 3 units of enzyme under oil incu-  
bated for 16 hours resulted in the same pattern of  
DNA bands as a reaction incubated for 1 hour with  
1 unit of enzyme.

**Exonuclease Activity:** Incubation of 50 units of  
enzyme with 1 µg sonicated [<sup>3</sup>H] DNA (10<sup>5</sup> cpm/µg)  
for 4 hours at 75°C in 50 µl reaction buffer released  
< 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 5 units of  
enzyme with 1 µg φX174 RF I DNA for 4 hours at  
75°C in 50 µl reaction buffer resulted in < 10%  
conversion to RF II.

### Enzyme Properties

#### Activity in NEBuffers:

NEBuffer 1	50%
NEBuffer 2	75%
NEBuffer 3	50%
NEBuffer 4	100%

When using a buffer other than the optimal  
(supplied) NEBuffer, it may be necessary to add  
more enzyme to achieve complete digestion.

**Survival in a Reaction:** A minimum of 0.13 unit  
is required to digest 1 µg of substrate DNA in  
16 hours.

**Heat Inactivation:** No.

**Notes:** TspMI is an isoschizomer of XmaI.

Temperature activity curve of activity:  
37°C gives 20% optimal activity  
65°C gives 100% optimal activity  
75°C gives 100% optimal activity  
80°C gives 200% optimal activity

Conditions of low ionic strength, high enzyme  
concentration, glycerol concentration > 5% or  
pH > 8.0 may result in star activity.

= Time-Saver™ Qualified (See www.neb.com for details).

CERTIFICATE OF ANALYSIS

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