# MspJI

NEW ENGLAND BioLabs Inc.
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www.neb.com

R0661S 002120614061

# R0661S

RR NEB4 BSA 37° Yes

| | | |
|---|---|---|
| **200 units** | **4,000 U/ml** | **Lot: 0021206** |
| **RECOMBINANT** | **Store at –20°C** | **Exp: 6/14** |

**Recognition Site:**

5′...$^{m}$C N N R (N)$_{9}$▼...3′
3′... G N N Y (N)$_{13}$▲...5′

**Description:** MspJI, an EpiMark®, validated product is a modification-dependent endonuclease that recognizes $^{m}$CNNR sites and generates a double-stranded DNA break on the 3′ side of the modified cytosine at $N_{12}/N_{16}$. The recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC) (1).

This enzyme is provided with an Enzyme Activator Solution which may be used for efficient digestion by MspJI.

The most common epigenetic modifications found in eukaryotic organisms are methylation marks at CpG or CHG sites. A subset of these modified sites are recognized and cleaved by MspJI.

At fully methylated CpG sites:

5′...Y N $^{m}$C G N R...3′
3′...R N G $^{m}$C N Y...5′

or CHG sites:

5′...Y $^{m}$C H G R...3′
3′...R G D $^{m}$C Y...5′

R = A or G
Y = C or T
H = A or C or T (not G)
D = A or G or T (not C)

MspJI recognizes each hemi-methylated site individually and cleaves bidirectionally to generate 32 base or 31 base fragments, respectively. These fragments contain the central methylated site and have 4-base 5′ overhangs at each end. MspJI does not cleave unmodified DNA.

**Source:** An *E. coli* strain that carries the synthetic MspJI gene from *Mycobacterium* species JLS

Supplied in: 300 mM NaCl, 10 mM Tris-HCl (pH 7.4 @ 25°C), 0.1 mM EDTA, 1 mM dithiothreitol, 200 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:**
10X NEBuffer 4, 30X Enzyme Activator Solution, 100X BSA

**Reaction Conditions:** 1X NEBuffer 4, supplemented with Enzyme Activator Solution and BSA. Incubate at 37°C.

**1X NEBuffer 4:**
50 mM potassium acetate
20 mM Tris-acetate
10 mM magnesium 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 pBR322 (dcm$^{+}$) DNA in 1 hour at 37°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 Assurance:** The EpiMark suite of products have been validated for use in an epigenetic application.

**Usage Notes:** Use of excess enzyme inhibits cleavage. Optimization of the amount of enzyme needed for complete digestion may be required for each substrate DNA. Excess of enzyme or prolonged digestion time in the presence of Enzyme Activator Solution may cause star activity.

**Protocol for Genomic DNA Digestion:**

1. Set up the following reaction in a sterile micro-centrifuge tube (it is important to add MspJI last):

| | |
|---|---|
| DNA (0.5 to 1 µg) | 1–5 µl |
| 10X NEBuffer 4 | 3 µl |
| BSA | 1 µl |
| 30X Enzyme Activator Solution | 1 µl |
| MspJI | 0.5–1 µl ( 2 to 4 units) |
| Nuclease-free water | to 30 µl |

2. Incubate at 37°C for 4–8 hours.

*(see other side)*

CERTIFICATE OF ANALYSIS

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This enzyme is provided with an Enzyme Activator Solution which may be used for efficient digestion by MspJI.
The most common epigenetic modifications found in eukaryotic organisms are methylation marks at CpG or CHG sites. A subset of these modified sites are recognized and cleaved by MspJI.
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or CHG sites:
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R = A or G
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*(see other side)*
CERTIFICATE OF ANALYSIS

## Quality Control Assays

**16-Hour Incubation:** A 50 µl reaction containing 1 µg of unmethylated substrate [pBR322 (dcm–)] and 40 units of enzyme incubated for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**Exonuclease Activity:** Incubation of 40 units of enzyme with 1 µg of a mixture of single and double-stranded $^{3}$H *E. coli* DNA ($10^{5}$ cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

**RNase Assay:** Incubation of a 10 µl reaction containing 20 units of MspJI with 40 ng of RNA transcript for overnight at 37°C resulted in no detectable degradation of the RNA as determined by agarose gel electrophoresis.

## Enzyme Properties

**Activity in NEBuffers:**

| | |
|---|---|
| NEBuffer 1 | NR |
| NEBuffer 2 | NR |
| NEBuffer 3 | NR |
| NEBuffer 4 | **100**% |

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

**Heat Inactivation:** 65°C for 20 minutes

### References


1. Zheng, Y. et al. (2010) *Nucl. Acids Res.* doi:10, 1093/nar/gkq327.
2. U.S. Publication No. 2010-0167942







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