McrBC

500 units 10,000 U/ml Lot: 0231206

RECOMBINANT Store at −20°C Exp: 12/12

Recognition Site:
5′...Pu mC (N40-3000) Pu mC...3′

Description: McrBC is an endonuclease which cleaves DNA containing methylcytosine* on one or both strands. McrBC will not act upon unmethylated DNA (1). Sites on the DNA recognized by McrBC consist of two half-sites of the form (G/A)mC.

Source: The two component proteins are purified separately from E. coli K-12 strains containing plasmids encoding McrB and McrC (1).

Applications:
• CpG methylation status: McrBC is a tool for determining the methylation state of CpG dinucleotides (6–10). McrBC will act upon a pair of Pu mC sequence elements, thereby detecting a high proportion of methylated CpG sites, but will not recognize Hpa II/Msp I sites (CCGG) in which the internal cytosine is methylated.
• Detection of cytosine-methylated DNA: The very short half-site consensus sequence (Pu mC) allows a large proportion of the methylcytosines present to be detected. Even DNA which is not heavily methylated can be detected, as a low level of cleavage occurs even when the Pu mC elements are as far as 3 kb apart.

Quality Control Assays
16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA (HindIII digest) and 20 units of McrBC for 16 hours at 37°C resulted in a DNA pattern free of detectable nuclelease degradation as determined by agarose gel electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction containing 50 units of McrBC with 1 µg of φX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

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*5-methylcytosine or 5-hydroxymethylcytosine or N4-methylcytosine (5).

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Heat Inactivation: 100 units of enzyme were inactivated by incubation at 65°C for 20 minutes.

Notes: McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base (2).

Therefore the enzyme does not produce defined DNA ends upon cleavage. Also, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA) the flexible nature of the recognition sequence results in an overlap of sites, and so a smeared rather than a sharp banding pattern is produced.

McrBC cleavage of the supplied 4.3 kb linear, methylated control plasmid DNA produces several fragments between approximately 700 bp and 2.3 kb in size.

GTP is more labile than other nucleotides. We recommend aliquoting the 100 mM solution supplied and thawing and diluting as necessary.

References:

U.S. Patent No. 5,405,760