McrBC

500 units 10,000 U/ml Lot: 0231304
RECOMBINANT Store at –20°C Exp: 10/13

Recognition Site:
5’...Pu mC (N4-3000) 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)

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Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 µg of a plasmid containing multiple McrBC sites in 1 hour at 37°C in a total reaction volume of 50 µl. A pilot titration of enzyme is recommended for cleavage of genomic DNA.

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 nuclease degradation as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction containing 25 units of McrBC with 1 µg of a mixture of single and double-stranded [3H] E. coli DNA (106 cpm/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

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.

(see other side)
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