McrBC

1X NEBuffer 2, supplemented with 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 nuclelease 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 [1H] E. coli DNA (100 cpmp/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.

McrBC

1X NEBuffer 2, supplemented with 100 µg/ml BSA, 1 mM GTP. Incubate at 37°C.

Reaction Conditions: 1X NEBuffer 2, supplemented with 100 µg/ml BSA, 1 mM GTP. Incubate at 37°C.

Reagents Supplied with Enzyme: 10X NEBuffer 2, 100X (100 mM) GTP, 100X BSA, Control Plasmid DNA.

Endonuclease Activity: Incubation of a 50 µl reaction containing 25 units of McrBC with 1 µg of a mixture of single and double-stranded [1H] E. coli DNA (100 cpmp/µg) for 4 hours at 37°C released < 0.1% of the total radioactivity.

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(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