Incubation of a 50 µl reaction containing 50 units of McrBC for 4 hours at 37°C released a mixture of single and double-stranded \([\text{H}]\) E. coli DNA (10^7 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 \(\phi X174\) 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