6143 North 60th Street
Milwaukee, WI 53218
www.chimerx.com

Certificate of Analysis

Telephone: (414) 535-9506
Toll Free: (800) 626-7833
Fax: (414) 535-9508
Web Support: chimerx.com/support
Email: info@chimerx.com

CviJ I *

Catalog No: 2126

Lot No: See Product Label
Package Size: See Product Label
Concentration: See Product Label
Source: Chlorella virus IL-3A
Storage Conditions: Store at -80°C
Notes: (1) CviJ I* restriction endonuclease is inhibited by glycerol concentrations in excess of 2.5%. Therefore the extension of the digestion time is recommended rather than using additional units of CviJ I*. Alternatively, DNA sample can be ethanol precipitated and re-digested.

(2) Due to extreme frequency of CviJ I*/ CviJ I recognition sites, sterical interference of closely located recognition sites is observed. It results in slower digestion of such sites. In consequence, the generated oligonucleotide fragments are rarely shorter than 15 bp that makes them ideal for anonymous primer applications.

(3) CviJ I* reaction buffer contains DMSO, which does not interfere with further enzymatic manipulations (ligations, labeling, etc). If the sample is intended for electrophoresis, ethanol precipitatation of the reaction mixture after completed digestion is strongly recommended in order to avoid diffused bands on agarose or polyacrylamide gels.

Recognition/Cut Site

5'-G↓C-3' except 5'-PyGCPu-3'
3'-C↑G-5' 3'-PuCGPy-5'

Description

- CviJ I* is an unique restriction enzyme capable of digesting DNA at two or three base recognition sequence (1,2)
- CviJ I (Cat. No. 2125-01) normally cleaves the sequence 5'...PuGCPy...3' between the G and C to leave blunt ends
- Under "relaxed" conditions (in the presence of Mg2+, ATP and enhancers), CviJ I* cleaves the sequences 5'...GC...3' except 5'...PyGCPu...3'
- Capable of cleaving single-stranded DNA and double-stranded DNA into small 20-200 bp fragments
- Generates numerous sequence specific oligonucleotides from unknown DNA samples

Applications

- CviJ I and CviJ I* cleave DNA extremely frequently and thus can be used for a variety of novel molecular biology applications (2,3,4)
- CviJ I / CviJ I* partial digests can also be used in applications such as shot-gun cloning, generating quasi-random libraries (2) and epitope mapping or panning
- CviJ I digestion of anonymous DNA produces a large number of oligonucleotide sized polymers upon thermal denaturation, which can be exploited in applications such as:

1. Large- scale mapping or sequencing projects utilizing anonymous primers
2. High resolution mappping of short DNAs
3. Nucleic acid labeling (Thermal Cycle Labeling, 4, Fig. 1)
4. Detection (5)
5. Amplification (5)
6. Cloning (2, 3)
7. Capture of nucleic acids

Unit Definition

One unit is the amount of enzyme required to completely digest 1 µg of pBR322 DNA in 1 hour at 37°C in a total reaction volume of 25 µl.

Heat Inactivation

10 minutes at 65ºC

Continued on back…

Reaction Temperature

37ºC

Assay Conditions

20 mM glycylglycine-KOH (pH 8.5)
0.1 mM dithiothreitol
50 mM potassium acetate
10 mM magnesium acetate
0.1 mM ATP and 30% DMSO
1 µg of pBR322
Incubation is at 37°C for 1 hour in a reaction volume of 25 µl

Storage Buffer

20 mM Tris-acetate (pH 8.0)
50 mM potassium acetate
0.5 mM EDTA
0.1 mM dithiothreitol
5 mM magnesium chloride
50% (v/v) glycerol

Reaction Buffer

200 mM glycylglycine-KOH (pH 8.5)
1.0 mM dithiothreitol
500 mM potassium acetate
100 mM magnesium acetate
1.0 mM ATP. DMSO is supplied separately

Quality Control

Endonuclease: Incubation of 2 units of CviJ I* with 0.25 µg of pBR322 DNA at 37°C for 16 hours (a 128-fold over-digestion) resulted in the same sharp characteristic banding pattern observed with 2 units of CviJ I* and 0.25 µg of pBR322 DNA in the standard 1 hour assay reaction, as determined by agarose gel electrophoresis. Reaction volume of 50 µl.

3'-Exonuclease: Incubation of 0.5, 1.0, and 2.0 units of CviJ I* with 5 pmoles of lambda/Taq I fragments (3'-labeled with Klenow exo- and [^{3}H]dCTP), incubated for 1 hour at 37°C resulted in ≤5% slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

5'-Exonuclease/5'-Phosphatase: Incubation of 0.5, 1.0, and 2.0 units of CviJ I* with 0.25 µg of [5'-^{32}P]lambda/Hae III fragments for 1 hour at 37°C resulted in ≤5% slope of %-end label released per unit of enzyme. Reaction volume of 50 µl.

References

(1) Xia, Y., Burbank, D., Uher, L., Rabussay, D. and Van Etten, J. Nucleic Acids Res.15, 6075-6090 (2) Fitzgerald,M.C., Skowron, P., Van Etten, J.L., Smith, L.M. and Mead, D.A. (1992) Nucleic Acids Res. 20,3753-3762 (3) Skowron, P.M, Swaminathan, N., McMaster, K., George, D., Van Etten, J. andMead, D. Gene 157 (1995) 37-41 (4) Mead, D., Swaminathan, N., Van Etten J. and Skowron, P.M.: Recombinant CviJI restriction endonuclease. (1995) Unites States Patent no US005472872A (5) Swaminathan, N., McMaster, K., Skowron, P. and Mead, D.A. Analytical Biochemistry 255 (1998) 133-141