MANUFACTURED BY EURx

roboklon

CviJI*

CviJ I*

Restriction Endonuclease

Recognition Sequence:

5`-G C-3" EXCEPT 5'-Py G C Pu-3'
3`-C G 5' 3'-Pu C G Py-5'

Cat. No.	Size
E2126-01	100 units
E2126-02	400 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 50°C

Prototype: CviJI*

Source: *Chlorella virus IL-3A*
Note 1: *Purified from a recombinant source (Patent No. US005472872A)*

Package Contents:
- CviJI*
- 2x Reaction Buffer CviJI*

Storage Conditions: Store at -20°C

Recommended Buffer: CviJI*

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.*

Customer note:

(1) CviJI* 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 CviJI*. Alternatively, DNA sample can be ethanol precipitated and re-digested.

(2) Due to extreme frequency of CviJI*/ CviJI 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) CviJI* 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.

Description:

CviJI* is an unique restriction enzyme capable of digesting DNA at two or three base recognition sequence (1,2). CviJI (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 Mg^{2+} , ATP and enhancers) CviJI* cleaves the sequences 5'-G C-3' except 5'-PyG CPu-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:

CviJI and CviJI* cleave DNA extremely frequently and thus can be used for a variety of novel molecular biology applications (2,3,4). CviJI 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) and **7.** capture of nucleic acids. CviJI/ CviJI* partial digests can also be used in applications such as shot-gun cloning, generating quasi-random libraries(2) and epitope mapping or panning.

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
12.5 µl 2x Buffer CviJI*
1-2 U CviJI* (use 1 U / µg DNA, < 10 % React. Volume!)
Tips: Add enzyme as last component. Mix components well before adding enzyme. After enzyme addition, mix gently by pipetting. Do not vortex.
Partial digestion yields random blunt-end DNA fragments for generation of randomized genomic libraries.
@ 25 µl H_2O, DNA and DNase free

Incubate for 1 h at 37°C

To obtain complete digestion of high molecular weight DNA, (e.g. plant genomic DNA), add excess amounts of enzyme and prolong the incubation time.

Stop reaction by alternatively
(a) Addition of 1.1 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation
20 min at 50°C *or*
(c) Spin Column DNA Purification
(e.g. EURx PCR/DNA CleanUp Kit, Cat.No. E3520) *or*
(d) Gel Electrophoresis and Single Band Excision
(e.g. EURx AgaroseOut DNA Kit, Cat.No. E3540) *or*
(e) Phenol-Chloroform Extraction or Ethanol Precipitation.

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 µg of Lambda DNA in 1 hr in a total reaction volume of 50 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x CviJI* Buffer: 20 mM glycylglycine-KOH (pH 8.5), 10 mM magnesium acetate, 50 mM potassium acetate, 0.1 mM ATP, 0.1 mM dithiothreitol, 30% DMSO.
Note 2: Reaction buffer is provided as 2x concentrated stock solution.

Storage Buffer:

20 mM Tris-acetate (pH 7.2 at 22°C), 0.5 mM EDTA, 0.1 mM dithiothreitol, 5 mM magnesium chloride, 50 mM potassium acetate, 50% (v/v) glycerol.

Quality Control:

Non-specific Endonuclease: Incubation of 1 unit of CviJI* with 1 µg of pBR322 plasmid DNA at 37°C for 16 hrs (a 160-fold over-digestion) resulted in the same sharp characteristic banding pattern as the standard assay reaction, as determined by agarose gel electrophoresis.
3'-Exonuclease: 0.3, 0.6 and 1.2 units of CviJI and 0.13 µg (0.65 pmol of 3'-ends) of lambda/TaqI fragments (3'-labeled with T4 DNA Polymerase and [^{3}H]dGTP and [^{3}H] dCTP), incubated for 1 hr at 37°C resulted in a 0.03 slope of %-end label released per unit of enzyme. Reaction volume 10 µl.
5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10 and 20 units of CviJI* with 0.05 µg (0.30 pmol of 5'-ends) of [5'-^{33}P]lambda/HaeIII fragments for 1 hr at 37°C resulted in a 0.024 slope of %-end label released per unit of enzyme. Reaction volume 10 µl.

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