EURx® MOLECULAR BIOLOGY PRODUCTS

CviJI*

5'-G C-3' EXCEPT 5'-Py G C Pu-3'
3'-C G-5' 3'-Py C G Pu-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: *Purified from a recombinant source (Patent No. US005472872A)*

Package Contents:

- **CviJI**
- **2 x Reaction Buffer CviJ***

Storage Conditions: Store at -20°C.

Unit Definition:

One unit is the amount of enzyme required to completely digest 1 µg of pBR322 DNA in 1 hour in a total reaction volume of 25 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.*

Description:

CviJI* is an unique restriction enzyme capable of digesting DNA at two or three base recognition sequence (1,2). CviJI (Cat. No. 2125) 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) CviI* cleaves the sequences 5'-G C-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.

Assay Conditions

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

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.

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.

Reaction Buffer:

1 x CviJI* Buffer: 20 mM glycylglycine-KOH (pH 8.5), 10 mM magnesium acetate, 50 mM potassium acetate, 0.1 mM dithiothreitol, 30% DMSO.

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 units of CviJI* with 1 µg of pBR322 plasmid DNA at 37°C for 16 hrs (a 16-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 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 0.024 slope of %-end label released per unit of enzyme. Reaction volume 10 µl.

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