

CVIJI 5'-Pu G C Py-3' 3'-Py C G Pu-5'

Cat. No.	size
E2125-01	100 units
E2125-02	400 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 50°C

Prototype: CviJI

Source: Chlorella virus IL-3A

Note: Purified from *E.coli* strain that carries the cloned *cvijRI* gene from *Chlorella* virus IL-3A (Patent No. US005472872A).

Package Contents:

- CviJl
- 5 x Reaction Buffer CviJI

Storage Conditions: Store at -20°C.

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.
- Mead, D., Swaminathan, N., Van Etten J. and Skowron, P.M.: Recombinant CviJI restriction endonuclease. (1995) Unites States Patent no US005472872A.
- 4.Skowron, P.M, Swaminathan, N., McMaster, K., George, D., Van Etten, J. and Mead, D. Gene 157 (1995) 37-41.
- 5.Swaminathan, N., McMaster, K., Skowron, P. and Mead, D.A. Analytical Biochemistry 255 (1998) 133-141.

Description:

CviJI recognizes two-three base pair sequence (1,2,3). This recombinant version of CviJI cleaves only PuGCPy sites (4). It does not exhibit star activity (CviJI*) (1,2), thus it is better suited for high resolution mapping of short DNA's like amplified products or small plasmids. Other CviJI applications, like shotgun cloning, thermal cycle labeling (5) or epitope mapping can be performed either with this version or with CviJI*.

Assay Conditions

20 mM glycylglycine-KOH (pH 8.5), 0.1 mM dithiothreitol, 50 mM potassium acetate, 10% DMSO, 10 mM magnesium acetate, 1 μ g of pBR322. Incubation is at 37°C for 1 hr in a reaction volume of 25 μ l.

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 $\mu l.$

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, 10% DMSO.

Storage Buffer:

20 mM Tris-acetate (pH 8.0 at 22°C), 50 mM potassium acetate, 0.5 mM EDTA, 3 mM dithiothreitol, 5 mM magnesium acetate and 50% (v/v) glycerol.

Quality Control:

Non-specific Endonuclease: Incubation of 10 units of CviJI with 1 μ g 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: 5, 10 and 20 units of CviJI and 0.13 μ g (0.65 pmol of 3'-ends) of lambda/Taql fragments (3'-labeled with T4 DNA Polymerase and [³H]dGTP and [³H] dCTP), incubated for 1 hr at 37°C resulted in 0.03 slope of %-end label released per unit of enzyme. Reaction volume 10 μ l.

5'-Exonuclease/5'-Phosphatase: Incubation of 5, 10 and 20 units of CviJI with 0.05 μ g (0.30 pmol of 5'-ends) of [5'-³³P]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 μ l.