

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

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.

Storage Conditions: Store at -20°C

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*.

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.

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.

Note 2: Reaction buffer is provided as 5x concentrated stock.

Assay Conditions:

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

Quality Control:

Non-specific Endonuclease: Incubation of 10 units 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: 5, 10 and 20 units of CviJI and 0.13 µg (0.65 pmol of 3'-ends) of lambda/TaqI 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 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'-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 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. Mead, D., Swaminathan, N., Van Etten, J. and Skowron, P.M.: *Recombinant CviJI restriction endonuclease.* (1995) *United 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.