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roboklon

CviJI

CviJ I

Restriction Endonuclease

Recognition Sequence:

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

Package Contents:
- CviJI
- 5x Reaction Buffer CviJI

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Relative Activity
Low	NR***
Medium	NR***
High	NR***
Acet	NR***

*** NR - buffer is not recommended, use 1 x buffer CviJII.

Recommended Buffer: CviJI

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 [^{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'-33P] 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.

Description:

CviJI is the only available restriction endonuclease recognizing two-three base pair sequence (1,2,3), thus allowing for new research applications (4). 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*.

Standard Reaction Protocol:

Mix the following reaction components:
- 1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
- 5 µl 5x 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. High (excess) amounts of enzyme can greatly speed up the reaction. 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 pBR322 DNA in 1 hour in a total reaction volume of 25 µl.

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 solution.

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:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, 5'-exonuclease/5'-phosphatase, as well as nonspecific single- and double-stranded DNase activities.

References:

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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.*
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