MANUFACTURED BY EURx

roboklon

BsiHKCI

BsiHKC I

Restriction Endonuclease

Recognition Sequence:

5`-C** Py C G Pu **G-3`
3`-G** Pu G C Py **C-5`

Cat. No.	Size
E2109-01	10 000 units
E2109-02	50 000 units

Reaction Temperature: 65°C

Inactivation Temperature (20 min): --

Prototype / Isoschizomer: Aval

Source: *Bacillus stearothermophilus HKC*
Note 1: *Purified from E.coli strain that carries the cloned bsiHKCRI gene from Bacillus stearothermophilus HKC.*

Package Contents:
- → BsiHKCI
- → 10x Reaction Buffer ONE
- → BSA [100x]
  Added as separate component to prevent reaction buffer precipitation.
- → Dilution Buffer # 1
  Added only for enzymes exceeding 10 U/µl in concentration. Use dilution buffer to dilute working stocks of enzyme to a customary concentration of 5 to 10 U/µl. Diluted enzyme stocks will not freeze during storage at -20°C.

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:
ONE Buffer is compatible with most EURx restriction enzymes.

DNA Methylation:
No inhibition: dam, dcm, EcoKI
Inhibition (Blocked):CpG

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 10x Buffer ONE
0.5 µl BSA [100x]
1-2 U BsiHKCI (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.
@ 50 µl H_2O, DNA and DNase free

Incubate for 1 h at 65°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 2.1 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation
(not applicable for this enzyme) *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.

Non-optimal buffer conditions:

To compensate for the lack of enzyme activity, increase the amount of enzyme and / or reaction time accordingly. The following values may serve as orientation:
- → *Enzyme amount:* Instead of 1 U enzyme, use ~4 U of enzyme in buffers providing 25 % rel. activity, ~2 U in 50 %, ~1.5 U in 75 % or ~1 U in 100 %, respectively.
- → *Reaction time:* Increase by ~1.3-fold (75 % rel. activity), ~2 fold (50 %) or ~4 fold (25 %), respectively.

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 ONE Buffer
To be supplemented with 100 µg/ml bovine serum albumin.

Reaction Buffer Compatibility:

Both, enzyme and buffers are fully compatible to restrictases and buffer systems from other manufacturers and can be used along in double digestions. To obtain best results, consult the corresponding manuals of all involved products.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 22°C), 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 500 µg/ml bovine serum albumin 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.