MANUFACTURED BY EURx

roboklon

EcoRI

EcoR I
Restriction Endonuclease

Recognition Sequence:

5`-G A A T T C-3`
3`-C T T A A G-5`

Cat. No.	Size
E2150-01	10 000 units
E2150-02	50 000 units
E2150-03	250 000 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: EcoRI

Source: *Escherichia coli RY 13*

Package Contents:
- → EcoRI
- → 10x Reaction Buffer ONE
- → BSA (100x) + detergent
  Added as separate component to prevent reaction buffer precipitation.
- → Dilution Buffer # 3
  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
Potential inhibition : 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) with Triton X100
1-2 U EcoRI (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 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 2.1 µl EDTA pH 8.0 (0.5 M), final 20 mM *or*
(b) Heat Inactivation
20 min at 65°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.

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.

Note 1: Addition of 0.025% detergen and BSA to reaction is required for optimal digestion.

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:

50 mM Tris_HCL (pH 7.2), 300 mM NaCl, 0.5 mM EDTA, 5 mM EGTA, 5 mM beta-mercaptoethanol, 0.2 % (v/v) Tergitol™ TMN, 500 µg/ml bovine serum albumin, 5 µg/ml PLL, 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.