DdeI

5'- C T N A G -3'
3'- G A N T C -5'

Cat. No.	Size
E2140-01	500 units
E2140-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 65°C

Prototype: DdeI

Source: *Desulfovibrio desulfuricans*

Package Contents:

- **DdeI**
- **10x Reaction Buffer High**
- **BSA [100x]**
  Added as separate component to prevent reaction buffer precipitation.
- **Dilution Buffer # 2**
  Added for enzymes exceeding 10 U/µl in concentration. High protein concentration warrants optimal stability during prolonged storage. Use dilution buffer to prepare short term working stocks (5-10 U/µl, non-freezing at -20°C).

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

Buffer	% Rel. Activity
Low	50
Medium	50
High	**100**
Acet	25

Recommended Buffer: High
(or compatible third party buffers)

Restriction Enzyme 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.

DNA Methylation:
No inhibition: dam, dcm, 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 High
0.5 µl BSA [100x]
1-2 U DdeI (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, nuclease 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:

1. *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.
2. *Reaction time*: Increase by ~1.3-fold (75 % rel. activity), ~2-fold (50 %) or ~4-fold (25 %).

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 High Buffer: 50 mM Tris-HCl (pH 7.5 at 37°C), 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol.
To be supplemented with 100 µg/ml bovine serum albumin.

Storage Buffer:

20 mM Tris-HCl (pH 8 at 22°C), 100 mM NaCl, 0.1 mM EDTA, 10 mM β-Me, 0,2% Triton X-100, 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, for nickase as well as for nonspecific single- and double-stranded DNase activities.