DpnI

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

Cat. No.	Size
E2135-01	500 units
E2135-02	2 500 units

Reaction Temperature: 37°C

Inactivation Temperature (20 min): 80°C

Prototype: DpnI

Source: *Diplococcus pneumoniae*
Purified from *E.coli* strain that carries the DpnI gene from *Diplococcus pneumoniae*.

Package Contents:

- **DpnI**
- **10x Reaction Buffer Acet**

Storage Conditions: Store at -20°C

Double Digestion Buffer Compatibility:

Buffer	% Rel. Activity
Low	100
Medium	100
High	75
Acet	**100**

The relative activity of DpnI in Pfu (Cat.No. E1114) and PfuPlus! (Cat.No. E1118) DNA Polymerase buffer is approx. 50 %.

Note 1: DpnI cleaves only methylated GATC sites.

Note 2: It may be necessary to add more enzyme to obtain complete digestion when using other buffer than optimal (Acet).

Note 3: It is recommended to add 10 U of the enzyme to obtain complete digestion of the methylated template after standard site-directed mutagenesis (total reaction volume is 50 µl).

Double digestions: 100 µg/ml BSA neither inhibits nor promotes DpnI cleavage.

Recommended Buffer: Acet
(or compatible third party buffers)

DNA Methylation:
No inhibition: All GATC sites with N^6-Methyladenine.

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA or 10 µl PCR product (=~0.1-2 µg DNA)
3 µl 10x Buffer Acet
1-2 U DpnI (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.
@ 30 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 1.2 µl EDTA pH 8.0 [0.5 M], final 20 mM *or*
(b) Heat Inactivation
20 min at 80°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 pBR322 *dam* methylated DNA in 1 hr. Total reaction volume is 30 µl. Enzyme activity was determined in the recommended reaction buffer.

Reaction Buffer:

1 x Acet Buffer: 20 mM Tris-acetate (pH 7.5 at 37°C), 10 mM magnesium acetate, 50 mM potassium acetate, 1 mM dithiothreitol.

Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 25°C), 1 mM dithiothreitol, 400 mM KCl, 0.1 mM EDTA, 0.1 % Triton X-100, 200 µg/ml bovine serum albumin and 50 %(v/v) glycerol.

Quality Control:

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