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roboklon

DpnI

Dpn I

Restriction Endonuclease

Recognition Sequence:

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 / Isoschizomer: DpnI

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

Package Contents:
- DpnI
- 10x Reaction Buffer ONE

Note 1: **DpnI cleaves only methylated GATC sites.**

Storage Conditions: Store at -20°C

Double Digestion – Buffer Compatibility:

ONE Buffer is compatible with most EURx restriction enzymes.

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

Recommended Buffer: ONE
(or compatible third party buffers)

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

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

Standard Reaction Protocol:

Mix the following reaction components:
1-2 µg pure DNA with methylated GATC sites
3 µl 10x Buffer ONE
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, DNA and DNase free

Incubate for 1 h at 37°C
(Agarose-embedded, RE buffer equilibrated DNA: 16 h)

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:

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

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 ONE Buffer

Restriction Enzyme Compatibility:

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

Storage Buffer:

10 mM Tris-HCl (pH 7.4 at 4°C), 1 mM dithiothreitol, 400 mM NaCl, 0.1 mM EDTA, 0.1% [v/v] Tergitol™ TMN, 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.