RESTRICTION ENDONUCLEASE NAR I

ProductInformation

Product Number **R5259**

Store at 0°C to -20°C

Product Description

Product Summary
Recognition Sequence: 5'GG/CGCC'3
Activity: 10,000 units/ml
Cutting: 100%
Ligation: >90%
Recutting: >90%
No degradation detected with >20 units for 16 hrs.
Fold over digestion: 320 (20 units x 16 hrs.)
Package Size: 1000 units

Unit Definition:

One unit is the enzyme activity that completely cleaves 1 µg Ad2- DNA in 1 hr. at 37°C in a total volume of 25 µl of SA restriction enzyme buffer.

Specificity

Nar I recognizes the sequence GG/CGCC and generates fragments with 5'-cohesive ends.[1] Nar I is similar to Nae I in that it demonstrates marked site preferences on different substrate DNA's, e.g.λ , pBR322 and Ad2.

Comments:

Digestion Buffer SA is supplied as a 10x concentrate.

Information is not available for heat inactivation of Nar I.

Several cleavage sites on these DNA's are cleaved at extremely slow rates and complete digestion is obtained only with large excess of enzyme.[2] Nar I generates compatible ends to Acy I, Cla I, Hpe II, Mae II, Msp I, Sfu I and Taq I. Nar I is inhibited by 5'-methylcytosine in the sequence GG/^{m}CGCC.

Nar I Storage and Dilution Buffer Composition

20 mM Tris-HCl
50 mM NaCl
0.1 mM EDTA
1 mM dithioerythritol
50% (v/v) glycerol
pH 8.0

1x Digestion Buffer SA (B 7531) Composition for Nar I: 100% Digestion at 37°C.

33 mM Tris-acetate
66 mM Potassium acetate
10 mM Magnesium acetate
0.5 mM dithiothreitol
pH 7.9

Quality Control Testing

Absence of non-specific endonuclease activities:
1 µg Ad2-DNA is incubated for 16 hrs. in 50 µl of buffer SA with excess units of Nar I.

Ligation and recutting assay
Nar I fragments obtained by complete digestion of 1 µg Ad2-DNA are adjusted to pH 7.5 at 20°C. The Nar I fragments are then ligated with 1unit T4-DNA ligase at pH 7.5 at 4°C. A 10 µl reaction mixture, incubated for 16 hrs. at 4°C, contained: 0.3 unit T4-DNA ligase, 66 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM dithioerythritol and 1 mM ATP.

The degree of ligation and subsequent recutting with Nar I to yield the typical pattern of Ad2 x Nar I fragments is determined.

References

1. Kessler, C., et al., Gene, 92, 1 (1990).
2. Gluck, B., et al., unpublished results

2/99