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ProductInformation

RESTRICTION ENDONUCLEASE Nde II

Product No. R 5757

Store at 0 °C to -20 °C

Product Summary

Recognition Sequence: 5'/GATC'3 Activity: Minimum 5,000 units/ml

Cutting: 100% Ligation: >95% Recutting: >95%

No degradation detected with >20 units for 16 hrs. Fold over digestion: 320 (20 units x 16 hrs.) Package Size: 200 units, 1,000 units

Unit Definition

One unit is the enzyme activity that completely cleaves 1 μ g λ damDNA in 1 hr. at 37 °C in a total volume of 25 μ l of Buffer SP for restriction endonucleases.

Specificity

Nde II recognizes the sequence /GATC and generates fragments with 5'-cohesive ends.¹

Comments

Digestion Buffer SP is supplied as a 2x concentrate.

Heat inactivation information is not available for Nde II.

Nde II Storage and Dilution Buffer Composition

20 mM Tris-HCl 50 mM NaCl 0.1 mM EDTA 1 mM dithioerythritol 0.02% Thesit (v/v) 50% (v/v) glycerol pH 7.5 Quality Control Testing 2x Digestion Buffer SP (B 6048) Composition for Nde II: 100% Digestion at 37 °C.

100 mM Tris-HCl 150 mM NaCl 10 mM MgCl₂ 1 mM dithiothreitol pH 7.6

Absence of unspecific endonuclease activities:

1 μ g λ damDNA or pBR322 DNA is incubated for 16 hrs. in 50 μ l buffer SP with excess of Nde II.

Ligation and Recutting Assay

Nde II fragments, obtained by complete digestion of 1 μ g λ DNA, are adjusted to pH 7.5 at 20 °C. The Nde II fragments are then ligated with 1.0 unit T4-DNA ligase at pH 7.5 at 4 °C. A 10 μ I reaction mixture, incubated for 16 hrs. at 4 °C, contained: 1.0 unit T4-DNA ligase, 66 mM Tris-HCl, 5 mM MgCl₂, 1 mM dithioerythritol and 1 mM ATP.

The degree of ligation and subsequent recutting with Nde II to yield the typical pattern of λ dam x Nde II fragments is determined.

References

1. Watson, R., et. al., FEBS Lett., 150, 114 (1982).

2/00