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ProductInformation

RESTRICTION ENDONUCLEASE Ava II

Product No. **R 6004** Store at 0 °C to –20 °C

Product Summary

Recognition Sequence: 5'G/G(AT)CC3'

Activity: 5,000 units/ml

Cutting: 100% Ligation: >90% Recutting: >95%

No degradation detected with >10 units for 16 hrs.

Fold over digestion: 160 (10 units x 16 hrs.)

Package Size: 100 units

Unit Definition

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

Specificity

Ava II recognizes the sequence G/G(AT)CC and generates fragments with 5'cohesive ends. Ava II is inhibited by overlapping dcm-methylation and by the presence of a 5'-methylcytosine or 5'-hydroxymethylcytosine at either C nucleoside.

Comments

Digestion Buffer SA is supplied as a 10x concentrate. Heat inactivation information is not available for Ava II.

Ava II Storage and Dilution Buffer Composition

20 mM Tris-HCl 100 mM NaCl 0.1 mM EDTA 10 mM 2-mercaptoethanol 0.01%(v/v) Triton X-100 50% (v/v) glycerol pH 8.0

Quality Control Testing

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

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

Absence of unspecific endonuclease activities:

1 μg λDNA is incubated for 16 hrs. in 50 μl buffer SA with excess of Ava II.

Ligation and Recutting Assay

Ava II fragments, obtained by complete digestion of 1 μ g λ DNA, are adjusted to pH 7.5 at 20 °C. The Ava II fragments are then ligated with 0.1 unit T4-DNA ligase at pH 7.5 at 20 °C. A 10 μ I reaction mixture, incubated for 16 hours at 20 °C, contained 0.1 unit T4-DNA ligase, 66 mM Tris-HCl, 5 mM MgCl2, 1 mM ATP and 1 mM dithioerythritol.

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

References

1. Murray, K., et al., Biochem. J., **159**, 317 (1976).

8/99