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ProductInformation

RESTRICTION ENDONUCLEASE Ava I

Product Number **R 3379**

Storage Temperature 0 to –20 °C

Product Description
Recognition Sequence: 5' C/PyCGPuG 3'
Activity: 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: 250 units

Unit Definition
One unit is the enzyme activity that completely cleaves 1 µg λDNA in 1 hr. at 37 °C in a total volume of 50 µl of Buffer SB for restriction endonucleases.

Specificity
Ava I recognizes the sequence C/PyCGPuG and generates fragments with 5' -cohesive termini.[1]

Comments
Digestion Buffer SB is supplied as a 10x concentrate. Information for heat inactivation of Ava I is not available.

Ava I Storage and Dilution Buffer Composition
20 mM Tris-HCl
100 mM NaCl
0.1mM EDTA
10 mM 2-mercaptoethanol
0.01% (v/v) Triton X-100
50% (v/v) glycerol
pH 8.0

1x Digestion Buffer SB (B 8781) Composition for Ava I: 100% Digestion at 37 °C.
10 mM Tris-HCl
10 mM NaCl
5 mM $MgCl_2$
1 mM 2-mercaptoethanol
pH 8.0

Quality Control Testing
Absence of unspecific endonuclease activities:
1 µg λDNA is incubated for 16 hrs. in 50 µl buffer SB with excess of Ava I.

Ligation and Recutting Assay
Ava I fragments, obtained by complete digestion of 1 µg λ DNA, are adjusted to pH 7.5 at 20 °C. The Ava I fragments are then ligated with 0.3 units T4-DNA ligase at pH 7.5 at 20 °C. A 10 µl reaction mixture, incubated for 16 hours at 20 °C, contained 0.3 units T4-DNA ligase, 66 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM ATP and 1 mM dithioerythritol.

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

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

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