



Product Information

RESTRICTION ENDONUCLEASE Afl III

Product No. **R 1259**

Store at 0 °C to -20 °C

Product Summary

Recognition Sequence: 5'A/C(AG)(TC)GT3'
Activity: 5000 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: 100 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 25µl of Buffer SH for restriction endonucleases.

Specificity

Afl III recognizes the sequence A/C(AG)(TC)GT and generates fragments with 5' cohesive ends.¹

Comments

Digestion Buffer SH is supplied as a 10x concentration. Afl III is not heat inactivated after incubation of 100 units at 65 °C for 15 minutes.

Afl III Storage and Dilution Buffer Composition

20 mM Tris-HCl
400 mM NaCl
0.1 mM EDTA
7 mM 2-mercaptoethanol
50% (v/v) glycerol
pH 7.6

1x Digestion Buffer SH (B3657) Composition for Afl III: 100% Digestion at 37 °C.

50 mM Tris-HCl
100 mM NaCl
10 mM MgCl₂
1.0 mM dithioerythritol(DTE)
pH 7.5

Quality Control Testing

Absence of unspecific endonuclease activities:

1 µg λDNA is incubated for 16 hrs. in 50 µl buffer SH with excess of Afl III.

Ligation and Recutting Assay

Afl III fragments, obtained by complete digestion of 1µg λDNA, are adjusted to pH 7.5 at 20 °C. The Afl III fragments are then ligated with 0.5 units T4-DNA ligase at pH 7.5 at 4 °C. A 10 µl reaction mixture, incubated for 16 hrs. at 4 °C, contained: 0.5 units T4-DNA ligase, 66 mM Tris-HCl, 5 mM MgCl₂, 5 mM dithioerythritol and 1 mM ATP.

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

References

1. Whitehead, P., et al., J. Gen. Microbiol., **131**, 951 (1985)

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