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ProductInformation

RESTRICTION ENDONUCLEASE EcoR II

Product No. **R 1636**
Store at 0 to –20 °C

PRODUCT SUMMARY
Recognition Sequence: 5' CC(A/T)GG '3
Activity: 10,000 units/ml
Cutting: 100%
Ligation: >90%
Recutting: >90%
No degradation detected with >50 units for 16 hrs.
Fold over digestion: 800 (50 units x 16 hrs.)

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 Buffer SH for restriction enzymes.

Specificity
EcoR II recognizes the sequence CC(A/T)GG and generates fragments with 5'-cohesive termini.[1]

Comments
Digestion Buffer SH is supplied as a 10x concentrate.

EcoR II is heat inactivated after 15 minutes incubation at 65 °C.

EcoR II Storage and Dilution Buffer Composition
20 mM Tris-HCl
300 mM KCl
1 mM EDTA
1 mM dithioerythritol
50% (v/v) glycerol
pH 7

QUALITY CONTROL TESTING
1x Digestion Buffer SH (B 3657) Composition for EcoR II: 100 % Digestion at 37 °C.
50 mM Tris-HCl
100 mM NaCl
10 mM $MgCl_2$
1 mM dithioerythritol
pH 7.5

Absence of unspecific endonuclease activities:
1 µg Ad2 DNA is incubated for 16 hrs. in 50 µl buffer SH with excess of EcoR II.

Ligation and Recutting Assay
EcoR II fragments, obtained by complete digestion of 1 µg Ad2 DNA, are adjusted to pH 7.5 at 20 °C. The EcoR II fragments are then ligated with 0.1 unit T4-DNA ligase at pH 7.5 at 4 °C. A 10 µl reaction mixture, incubated for 16 hrs. at 4 °C, contained: 0.1 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 EcoR II to yield the typical pattern of Ad2 x EcoR II fragments is determined.

References
1. Bigger, C. H., et al., Nature New Biol., **244**, 7 1973.

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