Apa I

GGGCC^C AccuCut™ Restriction Endonuclease

• Cat. No. E-1141 2500 Units E-1142 10000 Units

Lot No.: 02I131491H8A3

· Supplied with Enzyme

10X AccuCut™vlolet Buffer : 1 mL 330mM Tris-acetate pH 7.9 100 mM Mg-acetate 660 mM K-acetate 10mM DTT 1X Dilution Buffer : 1 mL 10 mM pH 7 6 Tris-HCI 50 mM KCI 0.1 mM FDTA 1 mM DTT $200 \mu \text{ g/mL}$ Acetylated BSA 50% Glycerol

- . Store at -20 ℃.
- Unit definition : One unit of restriction endonuclease activity is defined as the amount of enzyme required to completely digest $1\mu g$ of substrate DNA in a total reaction volume of $50~\mu L$ in one hour using the AccuCutTM buffer provided. Incubations are performed in 1.5 mL tubes at the appropriate incubation temperature as indicated in the Product Profile

• Isoschizomer : Ppe I.

• Neoschizomer : PspOM I (G^GGCCC).

- Reactivity on methylated substrate DNA: Blocked by overlapping dcm methylation (Cm5CWGG):GGGCCCWGG.
- Ref)1. Gunthert, U., Trautner, T.A., (1984) Curr. Top. Microbiol. Immunol., vol. 108, pp. 11-22.
 2. Kong, H., Unpublished observations.
 3. Seurinck, J., Van de Voorde, A., Van Montagu, M., (1983) Nucleic Acids Res., vol. 11, pp. 4409-4415.
 4. Trautner, T.A., Unpublished observations

- · Source : Acetobacter pasteurianus.
- Concentration: 20 Units/uL
- Reaction Condition
 - 10X AccuCut™ vlolet Buffer
 - Incubate at 37°C.

Storage Buffer

20 mM pH 7.5, Tris-HCl
50 mM KCl
1 mM EDTA
10 mM 2-mercaptoethanol
50% Glycerol

• Heat inactivation :65 °C for 20 minutes.

Quality Control

· Overdigestion Assay :

No nonspecific activity was detected after incubation of 1 μq of λ DNA with 50 units of Apa I for 15 hours.

- * Conditions of low ionic strength, high enzyme concentration, glycerol concentration >5%, or pH >8.0 may result in star activity.
- · Nuclease Contamination Assay :

No altered pattern was detected after incubation of 1 μg of substrate DNA with Apa I in 50 μL reaction volume with the supplied AccuCutTM buffer overnight.

Ligation and Recutting Assay :

This assay is used to test for exonuclease activity that would degrade the termini of restriction fragments, resulting in inhibition of ligation and of subsequent digestion of ligated fragments. After 40-fold overdigestion with *Apa* I, 95% of the DNA fragments can be ligated and recut with *Apa* I.