**ApaI**

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**Reagents Supplied with Enzyme:**
10X NEBuffer 4, 100X BSA

**Reaction Conditions:** 1X NEBuffer 4, supplemented with 100 µg/ml BSA. Incubate at 25°C.

**Product Details:**
- **5,000 units**
- **50,000 U/ml**
- **Lot:** 0451207

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5'...GGGCC...3' 3'...GGGCC...5'

**Source:** An *E. coli* strain that carries the cloned Apal gene from *Acetobacter pasteurianus* sub. *pasteurianus* (ATCC 9432)

**Supplied in:** 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 500 µg/ml BSA and 50% glycerol.

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**16-Hour Incubation:** A 50 µl reaction containing 1 µg of DNA and 100 units of Apal incubated for 16 hours resulted in the same pattern of DNA bands as a reaction incubated for 1 hour with 1 unit of enzyme.

**Exonuclease Activity:** Incubation of 100 units of Apal with 1 µg sonicated +H DNA (10^4 cpm/µg) for 4 hours at 25°C in 50 µl reaction buffer released <0.1% radioactivity.

**Endonuclease Activity:** Incubation of 100 units of Apal with 1 µg φX174 RF I DNA for 4 hours at 25°C in 50 µl reaction buffer resulted in <20% conversion to RF II.

**Enzyme Properties**

**Activity in NEBuffers:**
- NEBuffer 1: 25%
- NEBuffer 2: 50%
- NEBuffer 3: 0%
- NEBuffer 4: 100%

**When using a buffer other than the optimal (supplied) NEBuffer, it may be necessary to add more enzyme to achieve complete digestion.**

**Survival in a Reaction:** Suitable for an extended or overnight digestion. Enzyme is active >8 hours.

**Heat Inactivation:** 200 units of Apal were inactivated by incubation at 65°C for 20 minutes.

**Plasmid Cleavage:** Number of units required to cleave 1 µg of supercoiled plasmid DNA in one hour: 1 unit.

**Notes:** Apal is an isoschizomer of Bsp120I, but yields a 3’ extension.

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**Quality Control Assays**

**Ligation:** After 10-fold overdigestion with Apal, >95% of the DNA fragments can be ligated with T4 DNA Ligase at a 5’ termini concentration of 1–2 µM at 16°C. Of these ligated fragments, >95% can be recut.

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