When DNA containing a 22 base 5’ extension is used as a substrate for RecJ, the resulting products are a mixture of DNA fragments that have blunt-ends, 5’ extensions (1–5 nucleotides) and recessed 5’ ends (1–8 nucleotides) (3). RecJ does not require a 5’ phosphate (3).

Source: RecJ is overexpressed and purified as a C-terminal fusion to MBP. MBP does not affect the catalytic activity of RecJ, but does enhance RecJ solubility (2).

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol.

Reagents Supplied with Enzyme:
10X NEBuffer 2
Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.
1X NEBuffer 2:
50 mM NaCl
10 mM Tris-HCl
10 mM MgCl₂
1 mM dithiothreitol
pH 7.9 @ 25°C

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.05 nmol TCA soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C.

Heat Inactivation: 65°C for 20 minutes.

Quality Control Assays
3’→5’ ss and ds Exonuclease Activity: No detectable 3’→5’ nuclease activity was observed when 30 units of RecJ was incubated with substrates containing either 3’ extensions or blunt-ends.

Endonuclease Activity: Incubation of 10 units of RecJ, with 1 µg φX174 for 4 hours at 37°C in a 50 µl reaction resulted in < 10% conversion to RF II.

Single-Stranded Endonuclease: Incubation of 50 units of RecJ, with 1 µg of φX174 Virion DNA for 4 hours at 37°C in a 50 µl reaction resulted in no decrease in the amount of closed circular DNA as determined by agarose gel electrophoresis.

References:
2. Lovett, S. and Whitaker, R. unpublished observations.
3. Whitaker, R. unpublished observations.

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