RecJ<sub>f</sub>

When DNA containing a 22 base 5´ extension is used as a substrate for RecJ<sub>f</sub>, 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<sub>f</sub> does not require a 5´ phosphate (3).

**Source:** RecJ<sub>f</sub> is overexpressed and purified as a C-terminal fusion to MBP. MBP does not affect the catalytic activity of RecJ<sub>f</sub> but does enhance RecJ<sub>f</sub> 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.

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1X NEBuffer 2:

- 50 mM NaCl
- 10 mM Tris-HCl
- 10 mM MgCl<sub>2</sub>
- 1 mM dithiothreitol

**pH** 7.9 @ 25°C

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Quality Control Assays

**3´ → 5´ ss and ds Exonuclease Activity:**

No detectable 3´ → 5´ nuclease activity was observed when 30 units of RecJ<sub>f</sub> was incubated with substrates containing either 3´ extensions or blunt-ends.

**Endonuclease Activity:** Incubation of 10 units of RecJ<sub>f</sub> 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<sub>f</sub> 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 Lovett, S. and Whitaker, R. unpublished observations.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 Whitaker, R. unpublished observations.