**Source:** The two component proteins are purified separately from *E. coli* K-12 strains containing plasmids encoding Endonuclease VIII and Uracil-DNA Glycosylase.

Supplied in: 50 mM KCl, 5 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 175 µg/ml BSA and 50% glycerol.

**Reagents Supplied with Enzyme:** 10X CutSmart™ Buffer.

**Reaction Conditions:** CutSmart or PCR Reaction Buffer. Incubate at 37°C.

**1X CutSmart Buffer:**
- 50 mM Potassium acetate
- 20 mM Tris-acetate
- 10 mM Magnesium acetate
- 100 µg/ml BSA
- pH 7.9 @ 25°C

**Unit Definition:** One unit is defined as the amount of enzyme required to nick 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil base, in 15 minutes at 37°C in a total reaction volume of 10 µl.

**Unit Assay Conditions:** 1X T4 DNA Ligase Buffer containing 10 pmol of flourescently labeled oligonucleotide duplex in 15 minutes at 37°C in a total reaction volume of 10 µl.

**Quality Control Assays**

**Functional Test (USER, Transformation Assay):**
A 10 µl reaction in ThermoPol Reaction Buffer containing 20 ng linearized pNEB206A, 100 ng of a 950 bp control PCR product and 1 unit of USER Enzyme was incubated for 15 minutes at 37°C followed by 15 minutes at 25°C. After transformation into ER2267 chemically-competent cells > 95% of colonies contained recombinant plasmid.

**Heat Inactivation:** No

**Notes On Use:** USER Enzyme is active in all commercial PCR buffers tested. It also has 100% activity in TE (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA).

**References:**

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