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 fluorescently 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 Thermopoll 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: