so that base-free deoxyribose is released (4,5).

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.

Reaction Conditions: PCR Reaction Buffer. Incubate at 37°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 fluorescein labeled oligonucleotide duplex in 15 minutes at 37°C in a total reaction volume of 10 µl.

Quality Control Assays

A standard USER reaction was performed as described in Appendix V of the USER Friendly Cloning Kit (NEB #E5500) manual [20 ng linearized pNEB206A, 1 µl USER Enzyme and 10 µl (100 ng) of a 950 bp control PCR product amplified using Taq DNA Polymerase and primers containing uracil, designed as recommended in the USER Friendly Cloning Kit manual]. After transformation into chemically-competent cells (NEB ER2267 at 5 x 10^6 c.f.u./µg pNEB206A), 50 µl of the 1 ml outgrowth was spread on Amp + Xgal + IPTG plates. Greater than 95% of colonies were white (i.e., contained recombinant molecules).

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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