Source: An E. coli strain that carries the cloned UGI gene from Bacillus subtilis bacteriophage PBS1.

Application: Uracil Glycosylase Inhibitor (UGI) inhibits uracil-DNA glycosylase (UDG). Since UDG remains partially active following heat treatment at 95°C, UGI can be used to prevent subsequent degradation of product DNA.

Supplied in: 50 mM KCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50% glycerol (pH 7.4 ± 25°C).

Reagents Supplied with Enzyme: 10X Uracil DNA Glycosylase Buffer. Incubate at 37°C.

1X Uracil DNA Glycosylase Buffer: 20 mM Tris-HCl 1 mM EDTA 1 mM dithiothreitol (pH 8.0 ± 25°C)

Unit Definition: One unit of UGI is defined as the amount of protein required to inhibit one unit of E. coli UDG in 1 hour at 37°C in a total reaction volume of 50 µl. One unit of UDG is the amount of enzyme which will catalyze the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA.

Unit Assay Conditions: 1X Uracil DNA Glycosylase Buffer; 1 unit of Uracil DNA Glycosylase, 0.2 µg [3H]-uracil DNA (10^4–10^5 cpm/µg) for 30 minutes at 37°C in a total reaction volume of 50 µl.

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ phage DNA in NEBuffer 1 and 30 units of UGI showed no degradation following overnight incubation at 37°C.

Exonuclease Activity: Incubation of 50 units for 4 hours at 37°C in 50 µl of assay NEBuffer 1 with 1 µg [3H] DNA (10^6 cpm/µg) released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 50 units of UGI with 1 µg of φX174 RF I DNA for 4 hours at 37°C in 50 µl NEBuffer 1 resulted in < 5% conversion to RF II.

Heat Inactivation: No

Note: UGI is extremely thermostable, retaining > 95% activity after boiling for 10 minutes.

References:

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