Thermolabile Uracil DNA Glycosylase (UNG, UDG)

Cat #: C-BSM2906

Size: 500 U

Storage: -20°C

Components

Components	Size
Thermolabile Uracil DNA Glycosylase (UNG, UDG) (1 U/µl)	500 µl

Product Introduction

Thermolabile Uracil DNA Glycosylase (UNG, UDG) is a recombinant thermostable uracil-DNA glycosylase (UDG) derived from cold-water fish and purified through multiple steps. Compared to regular UDG, the thermostable UDG is more temperature-sensitive and can function at room temperature, but becomes inactivated at temperatures above 50°C. This enzyme catalyzes the hydrolysis of uracil bases in DNA chains containing uracil, releasing free uracil. It is commonly used to eliminate aerosol contamination caused by amplification in PCR, qPCR, and RT-qPCR.

Enzyme Activity Definition

One unit (U) is defined as the amount of enzyme required to degrade 1 µg of uracil-containing dsDNA within 30 minutes at 25°C.

Inactivation condition:

Incubation at 50°C for 5 minutes.

Quality Control

Endonuclease activity:

After incubating 1 U of Thermolabile Uracil DNA Glycosylase (UNG, UDG) with 200 ng of supercoiled plasmid DNA at 37°C for 4 hours, analyze the samples using agarose gel electrophoresis. Less than 10% of the plasmid DNA should be converted into nicks or linear forms.

Non-specific nuclease activity:

After incubating 1 U of Thermolabile Uracil DNA Glycosylase (UNG, UDG) with 15 ng of double-stranded DNA fragment at 37°C for 16 hours, analyze the double-stranded DNA substrate using agarose gel electrophoresis. There should be no change in the substrate.

RNase activity:

After incubating 1 U of Thermolabile Uracil DNA Glycosylase (UNG, UDG) with total RNA at 37°C for 1 hour, analyze the samples using agarose gel electrophoresis. More than 90% of the RNA should remain intact.

Host DNA residual:

Using specific primers targeting E. coli 16S rDNA and fluorescence quantitative PCR, detect the residual E. coli genomic DNA after treating with 1 U of Thermolabile Uracil DNA Glycosylase (UNG, UDG). The residual E. coli host genomic DNA should be below 10 copies.

Single-stranded DNA endonuclease activity:

After incubating 1 U of Thermolabile Uracil DNA Glycosylase (UNG, UDG) with 1 pmol of single-stranded oligonucleotide (FAM-labeled) at 37°C for 16 hours, analyze the samples using capillary electrophoresis. The degradation rate should be less than 5%.

Instructions for use:

1. Prepare the PCR reaction mixture according to the following system:

Reagents	Volume	Final Concentration
10× PCR Buffer for Taq (Mg2+ Plus)	5 µl	1×
dUTP (10 mM)[a]	3 µl	0.6 mM
dCTP/ dGTP/ dATP (10 mM each)	1 µl (each)	0.2 mM
Template DNA	X ng	—
Primer 1 (10 µM)	1 µl	0.2 µM
Primer 2 (10 µM)	1 µl	0.2 µM
AbTaq DNA Polymerase (5 U/µl)	0.5 µl	0.05 U/µl
Thermolabile Uracil DNA Glycosylase (UNG, UDG)[b]	1 µl	0.02 U/µl
ddH2O	Up to 50 µl	—

a. Adjust the final concentration of dUTP between 0.2 and 0.6 mM according to experimental needs.

b. b. The recommended usage of Thermolabile Uracil DNA Glycosylase (UNG, UDG) in a 50 µl reaction system is generally between 0.1 and 1 U.

2. Reaction Procedure

Step	Temperature	Time	
Degradation of uracil-containing template	25°C	10 min	
UDG inactivation, template denaturation	95°C	3 min	
Denaturation	95°C	10 s	30~35 Cycles
Annealing	55-65°C	30 s	
Extension	72°C	30 s/kb	
Final extension	72°C	5 min	

Note: The PCR reaction procedure can be adjusted according to experimental requirements.

Notes:

Thermolabile Uracil DNA Glycosylase (UNG, UDG) exhibits activity in most PCR or RT-PCR systems. However, when using a custom PCR or RT-PCR system, it is recommended to test compatibility with the system before initial use.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.