Best Way for Immunology

Uracil DNA Glycosylase (UNG, UDG)

REF: YS0026

Storage Condition

-20℃

Components

Component	Amount
Uracil DNA Glycosylase (UNG, UDG)(5 U/μl)	200 μl

Description

Uracil DNA Glycosylase (UNG, UDG) is a recombinant uracil-DNA glycosylase derived from *Escherichia coli* and purified through multiple steps. This enzyme catalyzes the hydrolysis of uracil bases in DNA chains, releasing free uracil. It is commonly used to eliminate aerosol contamination caused by PCR amplification.

Definition of Activity Unit

The amount of enzyme required to degrade 1 μg of dsDNA containing uracil within 30 minutes at 37℃ is defined as one unit.

Inactivation Condition

Incubation at 95℃ for 2 minutes.

Quality Control Assays

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 5 U of Uracil DNA Glycosylase (UNG, UDG) incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of double-stranded DNA fragments and 5 U of Uracil DNA Glycosylase (UNG, UDG) incubated for 16 h at 37℃ , and no degradation was detected by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of total RNA and 5 U of Uracil DNA Glycosylase (UNG, UDG) incubated for 1 hours at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the *E.coli* 16S rDNA , and the results show that the *E.coli* genome residues less than 10 copies.

Single-strand DNA (ssDNA) Nuclease Activity

After incubating 5 U of Uracil DNA Glycosylase (UNG, UDG) with 1 pmol of single-stranded oligonucleotide (labeled with FAM) at 37 ℃ for 16 hours, capillary electrophoresis was performed. The analysis showed that the degradation rate was less than 5%.

Protocol

1. Prepare the following reaction mixture on ice

Reagent	Amount	Final Concentration
10× PCR Buffer for Taq	5 μl	1×
dUTP (10 mM)[a]	1 μl	0.2 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)	0.2 μl	0.02 U/μl
Uracil DNA Glycosylase (UNG, UDG)[b]	0.2 μl	0.02 U/μl
ddH_2O	To 50 μl	-

a. The final concentration of dUTP may be adjusted between 0.2 to 0.6 mM.

b. In general, 0.1 ~ 1 U units of enzyme per 50 μl reaction is recommended.

2. PCR Reaction Program

Step	Temperature	Time	
UDG incubation	37℃	10 min	
UDG inactivation& Initial denaturation	95℃	5 min	
Denaturation	95℃	10 s	30~35 Cycles
Annealing	55~65℃	30 s	
Extension	72℃	30 s/kb	
Final Extension	72℃	5 min	

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

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