

Note: Usually, Taq enzyme and UNG enzyme are added to the PCR reaction system in a certain proportion, firstly digested at 37°C-20°C for 5-10 minutes, and then inactivated at 95°C for 10 minutes. (At the same time, this step also achieves pre-denaturation. and hot-start effect) followed by PCR amplification. The reaction of UNG enzyme can be varied in the range of 37°C-50°C, 5-10 minutes, which can be adjusted according to the needs of the experiment.

## Uracil-N-Glycosylase (UNG)

Cat. No. : CW0951S (200 U)  
CW0951M (1000 U)

Storage Conditions: -20°C

### Components

Component	CW0951S 200 U	CW0951M 1000 U
Uracil-N-Glycosylase, 1 U/μL	200 μL	1 mL

Note: The 10× PCR Buffer of this product contains 15 mM magnesium ions.

## Introduction

Uracil-N-glycosylase (UNGase), also known as uracil DNA glycosylase (UDGase), is a recombinant enzyme expressed and purified by *E. coli*. The protein has a molecular weight of 25 kD and can catalyze uracil-containing Single- and double-stranded DNA releases free uracil and is inactive to RNA, and is mainly used to prevent contamination of PCR amplification products. The mechanism of action of the enzyme is as follows: in the PCR reaction, dUTP is used instead of dTTP, and all Ts in the amplified product fragments are replaced by U, forming a PCR amplification product containing dU bases. UNG enzyme can selectively break the glycosidic bond of U base in single-stranded and double-stranded DNA, degrade the DNA containing U in the reaction system, effectively eliminate the residual contamination of PCR products, and greatly reduce false positives caused by contamination of amplification products, thereby ensuring specificity and accuracy of amplification.

## Activity Definition

One unit is defined as the amount of enzyme required to catalyze 1 nmol of uracil from uracil-containing DNA in 60 minutes at 37°C.

## Quality Control

The purity detected by SDS-PAGE was more than 95%; no endonuclease or exonuclease activity was detected.

## Precaution

1. For long-term storage of UNG (infrequent use; less than 3 times a month), please store it at -70°C. For daily or weekly use, please store recommended to use in separate packaging for large packaging.
2. UNG can remove inadvertently contaminating U-DNA molecules before PCR reactions. A laboratory must use dUTP as one of dNTPs in all PCR reactions, so that all amplified products become U-DNA. If it is used alone for a certain detection, T-DNA will still accumulate, and this anti-contamination system is difficult to play a full role.

3. The UNG/dUTP system is an anti-contamination measure inside the PCR reagent. In order to prevent the contamination of PCR products, especially when the same fragment is repeatedly amplified in the laboratory, the division and operation of the laboratory must be strictly regulated.

## Procedure

The following example is the use method of Taq reaction system to prevent PCR product contamination, and the practical application can be improved and optimized according to specific experiments.

### 1. PCR Reaction System

Reagent	50 µL	Final Conc.
Taq PCR Buffer, 10×	5 µL	1×
dATP, 10 mM	1 µL	200 µM
dGTP, 10 mM	1 µL	200 µM
dCTP, 10 mM	1 µL	200 µM
dTTP, 10 mM	0.5 µL	100 µM
dUTP, 10 mM	1 µL	200 µM
Forward Primer, 10 µM	1 µL	0.2 µM
Reverse Primer, 10 µM	1 µL	0.2 µM
Template DNA	X µL	
Taq DNA Polymerase, 5 U/µL	0.5 µL	2.5 U/50 µL
Uracil-N-Glycosylase, 1 U/µL	0.2 µL	0.2 U/50 µL
ddH <sub>2</sub> O	up to 50 µL	

### 2. PCR Reaction Condition

Step	Temperature	Time
UNG digestion	37-50°C	5-10 min
predenaturation	95°C	10 min
denaturation	94°C	30 s
annealing	55-65°C	30 s
extend	72°C	1 kb/min
final extension	72°C	5 min