Heat Labile UDG

For research use only

Components

Component	R5001 (500 U)	R5002 (100 U)
Heat Labile UDG (1 U/μl)	500 μl	100 μl

Storage

This reagent should be kept at -20°C.

Description

Heat Labile UDG is purified from the expression product of an recombinant *E. coli* strain containing the UDG gene cloned from cold-adaptive marine bacteria (psychrophilic Marine bacterium). UDG (Uracil-DNA Glycosylase) catalyzes the hydrolysis of uracil bases of the single or double stranded DNA containing dU and the N-glycoside bonds of the glycosphosphoric acid skeleton to release free uracil. The resulting base-free site is easily fractured by hydrolysis. This product is sensitive to high temperature, and above 50°C can make the enzyme irreversible inactivation, suitable for PCR, qPCR, RT-PCR, RT-qPCR system.

Storage Buffer

20 mM Tris-HCl, pH 7.5 at 25°C
0.1 mM EDTA
100 mM KCl
1 mM DTT
Glycerol 50% (v/v)
CA630 0.5% (v/v)
0.5% Tween - 20 (v/v)

Source

Psychrophilic Marine Bacteium derived Heat Labile UDG expressed and purified in *E. coli.*

Unit Definition

In 70 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1 mM EDTA, 100 g/ml BSA reaction solution, the amount of enzyme required to release 1 nmol uracil from DNA containing dU within 1 h at 37°C was defined as 1 unit of activity (U).

Thermal inactivation

50°C, 10 min; or 95°C, 2 min.

Protocol

By adding 1U Heat Labile UDG to 0.1 μg DNA containing uracil and reacting at 25°C for 10 minutes, the DNA would not be amplified by DNA polymerase.

1. Prepare the PCR reaction system as follows:

Component	Amount
10 × Taq Buffer (Mg^{2+} plus)	5 μl
dUTP[a]	0.6 mM
dATP/dCTP/dGTP	0.2 mM each

Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA	x μl
Taq DNA Polymerase (5 U/μl)	0.5 μl
Heat Labile UDG (1 U/μl)[b]	1 μl
ddH_2O	to 50 μl

a. According to the needs of the experiment, the final dUTP concentration can be adjusted between 0.2-0.6 mM.

b. According to the needs of the experiment, the amount of 50 μl reaction system is generally 0.1-1 U.

* According to the experimental needs, the final concentration of Mg^{2+} can be adjusted between 2-3 mM.

2. Reaction procedure

Temperature	**Time**	**Description**
25°C	10 min	degradation of U - containing templates
95°C	2 min	UDG inactivation, template denaturation
94°C	30 sec	30~35 cycles
55°C	30 sec	
72°C	60 sec/kb	
72°C	7 min	final extension

* The PCR reaction procedure can be adjusted according to the experimental needs.

Note

The activity of Heat Labile UDG reaches the optimal value at pH 8.0, does not require a divalent cation, and is inhibited at high ionic strength (> 200 mM).

Quality Control

Protein purity > 95% detected by SDS-PAGE.

Detection of no residue of endonuclease, DNase, RNase

Detection of no residue of *E.coli* DNA.