YTA Uracil-DNA Glycosylase (UDG)

For research use only

Cat No	Components	Conc	Size
YT9072	Uracil-DNA Glycosylase (UDG)	1U/µl	200U
	10X reaction buffer	-	0.4 ml

Description:

Uracil-DNA Glycosylase catalyzes the hydrolysis of the N-glycosylic bond between uracil and sugar, leaving an apyrimidinic site in uracil-containing single or double-stranded DNA. It releases uracil from ss- or ds- DNA and is applicable to eliminates PCR/qPCR carry-over contamination. This enzyme Shows no activity on RNA and oligomers(6 or fewer bases).

Applications:

- Control of carry-over contamination in PCR
- Enable Cloning of PCR products
- As a probe for protein-DNA interaction studies
- Glycosylase mediated single nucleotide polymorphism detection (GMPD)
- Site-directed mutagenesis
- Generation of single strand overhangs of PCR products and cDNA.

Definition of Activity Unit

One unit of the enzyme catalyzes the release 1 nanomole of uracil from uracil-containing DNA template in 60 min at 37 °C.

Storage buffer:

20 mM Tris-HCl (PH8) , 1 mM DTT, 0.1 mM EDTA, 100mM kCl , 50% Glycerol , 1% Triton x-100

10X Reaction buffer:

200 mM Tris-HCl, 10 mM DTT, 10 mM EDTA, pH 8 @ 25°C

Standard protocols:

- Replace dTTP in all amplification reactions by 600µM dUTP. when using dUTP the concentration of MgCl2 incresed up to 20Mm.
- Add 1µl of UDG(1U/µl) to 50µl RCR reaction and Incubate for 10 minutes at 37°C
- Inactive UDG by incubation at 95°C for 2-5min

Basic PCR protocol :

component	volume
10x Taq Buffer(with 20Mm MgCl2)	5µl
dUTP	0.6mM
dATP /dCTP /dGTP	0.2Mm each
Template DNA	optional
Primer1 (10µl)	2 µl
Primer 2(10µl)	2 µl
Taq DNA polymerase 5u/ul	0.5µl
UDG(1U/µl)	1µl
ddH20	To 50µl

In case of routine PCR:

step	Temperature	Time	Cycles
UDG activation	37°C	10min	1cycle
Pre-denaturation	95°C	2min	1cycle
denaturation	94°C	30sec	30-35 cycles
annealing	55°C	30sec	
extension	72°C	60sec	
Final extension	72°C	10min	1cycle