EZassay

Uracil DNA Glycosylase (UDG/UNG) heat-labile Instructions

info@ezassay.com
www.ezassay.com
EZassay Biotechnology Ltd.

Catalog Code: UG-HL-100
UG-HL-1000

CONTENTS

Product Information	
Product name	Uracil DNA Glycosylase (UDG/UNG), heat-labile
Quality	Recombinant protein
Form	Liquid

Storage

-20°C. Aliquot after receiving. Avoid repeated freeze-thaw.

Product Introduction

The UDG enzyme (Uracil-DNA Glycosylase) gene fragment is derived from psychrophilic marine bacteria. The UDG enzyme catalyzes the hydrolysis of the uracil base of the uracil-containing DNA strand and the N-glycosidic bond of the glycosphophosphate backbone, releasing free uracil, and the resulting base-free sites are easily broken by hydrolysis. This enzyme can act on single- or double-stranded DNA containing dUTP and is inactive against RNA. This product is sensitive to high temperature, and can irreversibly inactivate the enzyme above 50°C, and is suitable for LAMP, RT-LAMP, PCR, qPCR, RT-PCR, RT-qPCR systems

Materials supplied

Component	UG-HL-100	UG-HL-1000
UDG (heat-labile)*	100U	1000U

*Storage buffer： 10 mM Tris-HCl, pH 7.4@25°C, 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 50% Glycerol (v/v).

Unit definition

One unit is the amount of enzyme required to liberate 1 nmol uracil from dUTP-containing DNA in one hour at 37°C.

Inactivation

Inactivated by heating at 50 °C for 10 min.

Application examples

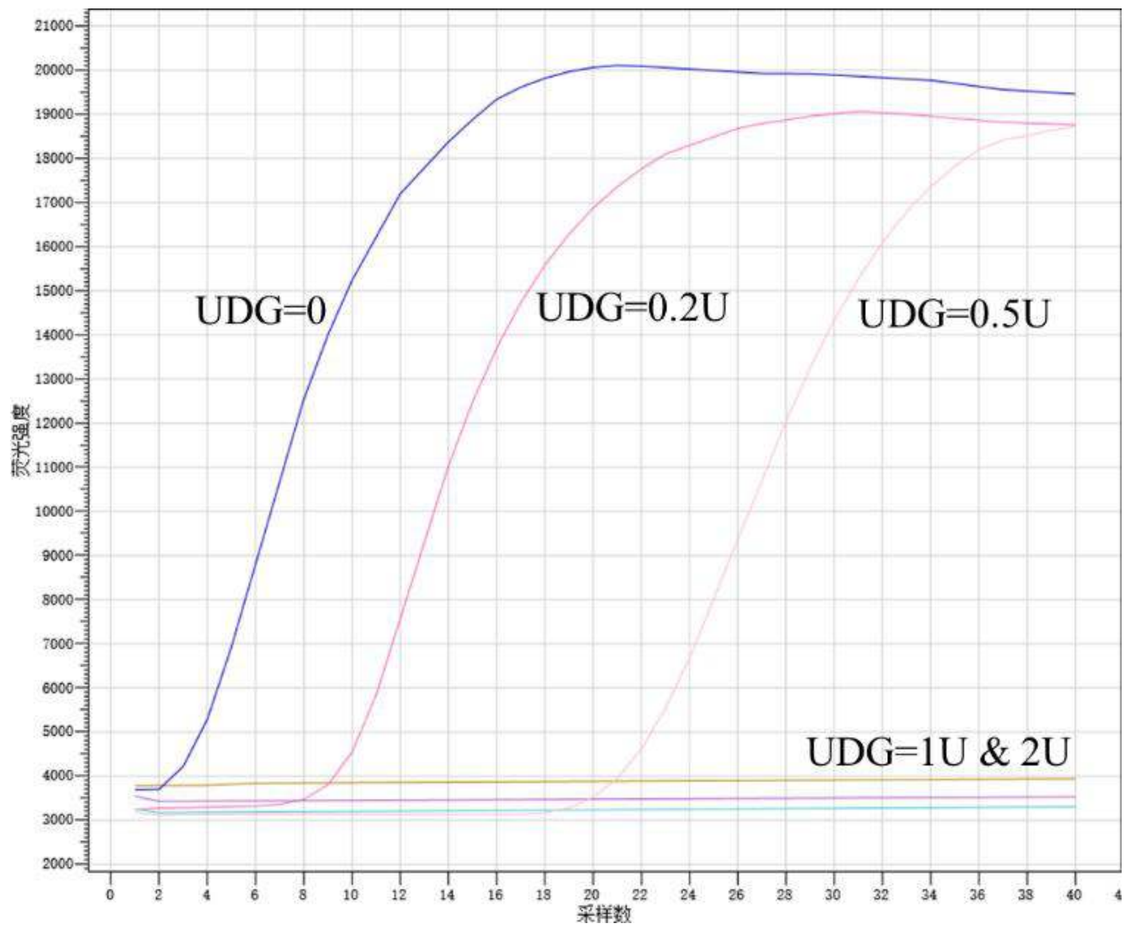
Application example ①：UDG in LAMP
1. Prepare LAMP reaction solution according to the following table:

Component	Volume	Working concentration
DNA template	1 µl	>10 copies or more
Primer mix (10X)	2.5 µl	1.6 µM FIP/BIP, 0.4 µM LF/LB, 0.2 µM F3/B3,
dUTP mix (10 mM each) *	2 µl	0.8 mM
Bst2.0 (8 U/µl)	1 µl	-
UDG (1 U/µl)	0.5~2 µl	-
10X Isothermal Amplification Buffer	2.5 µl	1X
100mM MgSO4	1.5 µl	6mM+2mM in 1X Isothermal Amplification buffer=8mM final
Nuclease-free H2O	Up to25 µl	-

*The final dUTP concentration can be adjusted between 0.2-1.4 mM according to experimental needs. Optional incorporation of 0.2 mM dTTP.

2. Incubate at 25°C for 10 minutes to allow UDG enzyme to degrade the U-containing template.
3. Incubate at 65 °C for 40 minutes to perform LAMP reaction.

Example of preventing contamination with UDG enzyme:



DNA template: amplicons containing dUTP. After diluting 10 times, take 2μl as template for LAMP reaction.
Result: UDG enzyme can effectively degrade amplicons containing dUTP. It can used to prevent false positive results caused by carryover contamination.

Application example②：UDG in PCR

1. Prepare PCR reaction solution according to the following table:

Component	Volume	Working concentration
DNA template	1 μl	>10 copies or more
Primer mix (F & R primer 10 μM each)	1 μl	0.4 μM each
dUTP (10 mM)*	1.5 μl	0.6 mM
dCTP / dGTP / dATP / dTTP (10 mM each)	0.5 μl	0.2 mM each
Taq DNA Polymerase (2.5 U/μL)	0.5 μl	-
UDG (1 U/μl)	1~2 μl	-
10X PCR Buffer (Mg2+ Plus)	2.5 μl	1X
Nuclease-free H2O	Up to 25 μl	-

*The final dUTP concentration can be adjusted between 0.2-1.4 mM according to experimental needs. Optional incorporation of 0.2 mM dTTP.

2. Set the program：

Temperature	Time	Cycles	Purpose
25°C	10 min	1	Degradation of dUTP-containing DNA
94°C	2 min	1	Inactivate UDG enzyme; Pre-denature template.
95°C 60°C 72°C	10 sec 20 sec 30 sec/kb	30-35	Denature template Anneal primers Extension
72°C	5 min	1	Final extension

3. Start the reaction.